The American Journal of Human Genetics

Vol. 13 No. 4 *December 1961*

Genetic and Population Studies of the Blood Types and Serum Factors Among Indians and Chinese from Malaya

ARTHUR G. STEINBERG[1], L. Y. C. LAI[2], G. H. VOS[3], R. BHAGWAN SINGH[4], AND T. W. LIM[4]

With the technical assistance of Janet A. Wilson and Dell Vos

WHILE THE PATTERN OF INHERTANCE and the distribution of the blood types among the various populations of the world are well known (Race and Sanger, 1958; Mourant, 1954; Mourant, Kopeć, and Domaniewska-Sobczak, 1958), neither the pattern of inheritance nor the distribution of the serum factors (Gm, Hp, and Tf) is clearly understood. Thus, there is considerable uncertainty with regard to the pattern of inheritance of the Gm and Hp groups in different populations (Steinberg, Stauffer, Blumberg, and Fudenberg, 1961; Giblett and Steinberg, 1960; also others) and little population data for each of the three serum factors are available. We have studied the families of Chinese and Indians in Malaya in the hope of contributing useful information concerning the serum factors. The blood types were studied primarily to detect extra-marital offspring. Families with only Indian or Chinese ancestry were selected.

MATERIALS AND METHODS

Venous blood samples were collected from parents and children of Chinese and Indian families in and around Kuala Lumpur, Malaya. The blood types, and the Hp and Tf types were determined at the University of Western Australia, the Gm types were done in duplicate in Perth and in Cleveland. The blood types were determined with the following anti-sera: anti-A, anti-B, anti-A+B, anti-A (seed), anti-M, anti-N, anti-C, anti-D, anti-E, and anti-c. All cells positive for E were tested with anti-c. The Hp and Tf types were determined by the vertical technique of starch gel electrophoresis (Smithies, 1959), using hydrolysed starch and borate buffer. The reagents used to determine the Gm types are presented in table 1. We are grateful to Dr. M. Harboe of the University of Oslo for the gift of the sera A. Berg and N. Berg, and to Dr. Tibor J. Greenwalt of the Milwaukee Blood Center for the gift of the sera Rh 7 and

Received July 7, 1961.

This investigation was supported in part by PHS grant RG 7214 from the Division of General Medical Sciences, Public Health Service and by a grant from the Research Grant Committee of the University of Western Australia.

[1] *Western Reserve University, Cleveland, Ohio.*

[2] *University of Western Australia, Nedlands, Western Australia.*

[3] *King Edward Memorial Hospital, Perth, Western Australia.*

[4] *Institute of Medical Research, Kuala Lumpur.*

TABLE 1. REAGENTS USED FOR GM TYPING

	For Original Typing			For Confirmatory Typing		
	Agglutinator	Anti-D	Dilution of Normal Sera	Agglutinator	Anti-D	Dilution of Normal Sera
	Cleveland, Ohio					
Gm(a)	Wils. 1/16	Kim. 1/10	1/8, 1/16	Wils. 1/16 Rh 7—1/16	Rh 251—1/10 Ham. 1/10	1/8, 1/16 1/8, 1/16
Gm(x)	Bowers 1/64	Ham. 1/10	1/8, 1/16	Bowers 1/64	Mag. 1/10	1/4, 1/8
Gm(b)	Bomb. 1/32	Pac. 1/10	1/8, 1/16	A. Berg 1/30	N. Berg 1/5	1/8, 1/16
Gm-like	Bomb. 1/32	Warren 1/10	1/16, 1/32	Carp. 1/16 Tube Test: Bomb. 1/32	Warren 1/5 Warren 1/10	1/16, 1/32 1/16, 1/32
	Perth, Australia					
Gm(a)	Higham 1/32	Ahearn 1/5	1/8, 1/16			
Gm(x)	Bowers 1/32	Rankin 1/5	1/8, 1/16			
Gm(b)	Bomb. 1/32	Gabriel 1/5	1/8, 1/16			
Gm-like	Bomb. 1/32	Warren 1/10	1/16, 1/32			

Rh 251. Rh 7 is unusual in that it is an anti-D serum which can be used as an anti-Gm(a) serum (agglutinator), but thus far has not proved useful as an anti-D in the Gm system. It may be used without absorbing the anti-D activity from it.

THE DATA AND DISCUSSION

The detailed family data are presented in the appendix table. There are 128 unrelated Indians among the 65 families (two families, I 25 and I 65 are represented by one parent each) and 90 unrelated Chinese among the 46 families (two families Ch 8 and Ch 47 are represented by one parent each).

The Blood Types

A summary of the ABO, MN, and Rh blood types among the parents is presented in table 2.

We are not aware of any previous study on the Indians living in and around Kuala Lumpur, hence we have no standards for comparison. However, the observed phenotypes seem to be consistent with a Hardy-Weinberg equilibrium. Thus the frequencies of I^A, I^B, i alleles (p, q, r, respectively) estimated by Bernstein's method, (without correction are: .175, .194, and .632, respectively. Their total exceeds one by only 0.001. The $\chi^2_{(1)}$ for the test of the fit of the MN data to the Hardy-Weinberg equilibrium, with M = .614, N = .386, is 1.335, hence $.3 > P > .2$. We have not analysed the data for the Rh blood types, since the sample is small, and we have made our point concerning the nature of the sample in considering the ABO and MN types.

TABLE 2. BLOOD TYPES OF THE PARENTS OF INDIAN AND CHINESE FAMILIES FROM MALAYA

(The cells were tested with the following anti-sera: anti-A, anti-B, anti-A + B, anti-A_1 (Seed), anti-M, anti-N, anti-C, anti-D, anti-E, and anti-e. All cells positive for E were tested with anti-e. For the Rh types, the positive reactions are recorded.)

	Total	ABO					MN			Rh								
		O	A_1	A_2	B	A_1B	M	MN	N	CD	CDEce	CDc	Cc	CDEc	DEc	DEce	Dc	c
A. Indians																		
No. 128		51	31	1	36	9	45	67	16	55	11	46	2	0	0	2	1	11
%		39.9	24.2	0.8	28.1	7.0	35.2	52.3	12.5	42.9	8.6	35.9	1.6	—	—	1.6	0.8	8.6
B. Chinese																		
No. 90		39	23	0	26	2	25[1]	43	22	41	33[2]	6	0	1[3]	7	2	0	0
%		43.3	25.6	—	28.9	2.2	27.8	47.8	24.4	45.5	36.7	6.7	—	1.1	7.8	2.2	—	—

[1] Reaction of one sample with anti-N doubtful; scored as N negative.
[2] Three samples reacted weakly with anti-D; scored as D positive.
[3] Reaction with anti-C weak; scored as C positive.

The ABO blood groups of the Chinese agree well with the findings of Allen and Scott (quoted in Mourant *et al.*, 1958) on a sample of 624 Chinese from Singapore. They found the following frequencies: O = .431, A = .240, B = .277, and AB = .051. The frequencies of the ABO phenotypes are similar also to those reported by Simmons, Graydon, Semple, and Green (1950) on samples taken from three Chinese populations in Malaya.

The frequencies of the M allele varied among the three populations sampled by Simmons *et al.*, from .489 in the Hokkiens to .670 in the Cantonese. The frequency in our sample is .517, i.e., close to that of the Hokkiens. Our sample shows a satisfactory fit to the values expected in a population in Hardy-Weinberg equilibrium ($\chi^2_{(1)} = 0.162, .7 > P > .5$).

Simmons *et al.* (1950) tested their samples with the same five Rh anti-sera that were used on our samples. In general, the two sets of data are in agreement in that both samples show a high frequency of the R_1R_1 and R_1R_2 phenotypes, the presence of R_Z, and an absence of the Rh negative phenotype.

The blood types indicate extra-marital children in families I 4 (child b, by the ABO groups), I 11 (child b, by the ABO groups), Ch 24 (child a by the Rh types and child b by the MN types), and possibly in Ch 25 (child a by the Rh type; this child is excluded by the Hp types, see below).

Haptoglobin and Transferrin Types

A summary of the Hp and Tf types of the two sets of parents is presented in table 3. Kirk, Lai, Mahmood, and Singh (1960) have published data on the frequency of haptoglobins in populations of South-East Asia. These data show

TABLE 3. HAPTOGLOBIN AND TRANSFERRIN TYPES OF INDIANS AND CHINESE FROM MALAYA

	Total	Haptoglobins				Transferrins	
		1-1	2-1	2-2	0	CC	CD_1
			A. Indians				
No.	128	0	23	102	3	128	0
%		—	18.0	79.7	2.3	100.0	—
			B. Chinese				
No.	90	9	32	46	3	86	4
%		10.0	35.6	51.1	3.3	95.6	4.4

that both Indians and Chinese have a low frequency of the Hp^1 allele, .09 for Indians, and .28 for the Chinese. In our samples the frequency of Hp^1 is .09 for the Indians and .29 for the Chinese.

The family data shed no light on the pattern of inheritance of the Hp O phenotype. Giblett and Steinberg (1960) have suggested that a third allele, Hp^{2M}, when heterozygous with Hp^1 may yield either the phenotypes Hp 2-1M or Hp O. The genotype $Hp^{2M}Hp^2$ is probably Hp 2-2. Unfortunately we do not know whether any of the ahaptoglobinemic individuals is so for genetic reasons or because he is suffering from a hemolytic disease. At any rate, among the Indians, families 1, 23, and 54 each have one parent who is Hp O. Each of these families is consistent with Giblett and Steinberg's hypothesis described above. In family 57, however, we find an ahaptoglobinemic child as the offspring of Hp 2-2 parents. If this child is not extra-marital or suffering from a hemolytic disease, it contradicts the above hypothesis.

Among the Chinese, families 6, 27, and 37 each have an Hp O parent, and each is consistent with the hypothesis of a third allele. In addition, families 1, 2, 32, 33, and 41 have Hp O children with neither parent Hp O. All of these except family 1 are consistent with the hypothesis.

Only transferrin C was found among the Indians, while among the Chinese about four per cent of the parents had Tf CD_1 ; the remainder had Tf CC. Incidentally, the transferrins indicate that child b in family Ch 25 is extra-marital; it has Tf CD_1 , while both its parents have Tf CC.

Our data may be compared with the data published by Lai (1961) on the Tf groups of Indians, Chinese, and Malayans from Malaya. In his sample one of 298 Indians had Tf BC, the remainder had Tf CC; one of 103 Chinese had Tf DD, seven had Tf CD, the remainder had Tf CC.

Gm Types

A summary of the Gm phenotypes of the two sets of parents is presented in table 4. The frequencies of the individual factors are presented in table 5 and summaries of the several types of matings are presented in tables 6 and 7. Both populations were completely negative for Gm-like.

The Indians have a high frequency of Gm(a) and of Gm(x), and a low frequency of Gm(b). The pattern of the frequencies of Gm factors is similar to that shown by North and South American Indians (Steinberg, *et al.*, 1961). The

TABLE 4. GM PHENOTYPES OF INDIANS AND CHINESE FROM MALAYA TESTED FOR GM(A), GM(X), GM(B), AND GM-LIKE

(All were Gm-like (−). Only the factors for which the sera were positive are indicated.)

	Total	Phenotype									
		ab		a		axb		ax		b	
		No.	%	No.	%	No.	%	No.	%	No.	%
Indians	128	30	23.4	39	30.5	17	13.3	36	28.1	6	4.7
Chinese	90	81	90.0	4	4.4	3	3.3	2	2.2	0	—

TABLE 5. FREQUENCIES (IN PER CENT) OF GM FACTORS IN INDIANS AND CHINESE FROM MALAYA

(All were Gm-Like Negative)

	Total	GM Factor		
		a	x	b
Indians	128	95.3	41.4	41.4
Chinese	90	100.0	5.5	93.3

TABLE 6. GM PHENOTYPES[1] AMONG THE MATINGS OF INDIAN FAMILIES FROM MALAYA. EACH FAMILY HAD TWO CHILDREN

(Families in which one or more offspring are excluded by blood groups, haptoglobins, transferrins, or Gm types are not included[2])

Mating[3]	n	Phentoypes of Offspring						Total
		a	ab	b	ax	axb	b	
a × ax	9	7	—	—	11	—	—	18
a × a	9	18	—	—	—	—	—	18
a × ab	8	8	8	—	—	—	—	16
ax × axb	7	—	2	—	8	4	—	14
ab × ab	5	1	6	3	—	—	—	10
ax × ab	5	—	6	—	2	2	—	10
ax × ax	4	1	—	—	7	—	—	8
ab × axb	3	—	3	1	1	1	—	6
a × axb	2	—	2	—	2	—	—	4
b × ab	2	—	2	—	—	—	2	4
axb × axb	1	—	—	—	—	2	—	2
b × ax	1	—	2	—	—	—	—	2
b × b	1	—	—	2	—	—	—	2

[1] All sera were tested for Gm(a), Gm(x), Gm(b), and Gm-like. Only the positive reactions are recorded in the table.

[2] See familes I 4, I 11, and I 15, in appendix. Three additional families (I 44, I 48, and I 58) are discussed separately.

[3] Phenotypes of parents are given without regard to sex of parent (i.e., a × ab = ab × a).

pattern differs in that the American Indians appear to be 100 per cent Gm(a+) and to have the Gm^{ab} allele postulated for Negroids by Steinberg, Stauffer, and Boyer (1960), while the eastern Indians are not 100 per cent Gm(a+) and do not seem to have the Gm^{ab} allele (tables 4, 5, and 6). The population data may be explained by assuming the presence of alleles Gm^{a}, Gm^{ax}, and Gm^{b}. The frequencies of these three alleles as determined by the maximum likelihood equations published by Steinberg *et al.* (1961) are .535, .234(5), and .230(5), respectively. The agreement between the observed and expected frequencies of

TABLE 7. GM PHENOTYPES[1] AMONG THE MATINGS OF CHINESE FAMILIES FROM MALAYA

(Families in which one or more offspring are excluded by blood groups, haptoglobins, transferrins, or Gm types are not included[2])

Mating[3]	s	n_s	Phentoypes of Offspring					Total
			a	ab	b	axb	ax	
ab × ab	1	5	0	5	0	—	—	5
	2	27	1	53	0	—	—	54
	3	1	0	3	0	—	—	3
a × ab	2	4	1	7	—	—	—	8
ab × axb	2	2	—	3	0	1	0	4
ax × axb	2	1	—	1	—	—	1	2
ab × ax	2	1	0	0	—	2	0	2

[1] All sera were tested for Gm(a), Gm(x), Gm(b), and Gm-like. Only the positive reactions are recorded in the table.

[2] See families Ch 24 and Ch 25 in appendix.

[3] Phenotypes of parents are given without regard to sex of parent (i.e., a × ab = ab × a)

the five phenotypes is satisfactory ($\chi^2_{(2)} = 1.336$; $.7 > P > .5$). The assumption of the presence of the alleles Gm^a, Gm^{ax}, and Gm^b in this population is supported by the family data (table 6), see especially matings of a × ax, a × ab, ax × axb, and ab × ab.

Three families (I 44, I 48, and I 58, see appendix) require special consideration, because they cannot be explained by assuming the presence of alleles Gm^a, Gm^{ax}, and Gm^b. In family I 44 the mating is Gm(a+ x+ b+) × Gm(a+ x− b−). We would expect the mother to be Gm^{ax}/Gm^b and the father to be Gm^a/Gm^a, but both children are Gm(a+ x+ b+). If the children are not extra-marital (We have no evidence to indicate that they are.), the mother's genotype must be Gm^{xb}/Gm^a or Gm^{axb}/Gm^a. The mating in family I 48 is

$$\text{Gm(a− x− b+)} \times \text{Gm(a+ x+ b+)},$$

i.e., expected genotypes $Gm^b/Gm^b \times Gm^{ax}/Gm^b$, but one child is Gm(a+ x− b+) and one is Gm(a+ x+ b+). If the ab child is not extra-marital, the father would have to be Gm^a/Gm^{xb} or Gm^a/Gm^{axb}. In family I 58 the mating is Gm(a+ x− b+) × Gm(a+ x+ b+), i.e., expected genotypes $Gm^a/Gm^b \times Gm^{ax}/Gm^b$, but both children are Gm(a+ x− b−). If they are not extra-marital the father would have to be Gm^a/Gm^{xb} *or* Gm^a/Gm^{axb}.

Henningsen (unpublished data) has unexceptionable data demonstrating the presence of the allele Gm^{xb} in a Danish family. In the light of his data and the data indicating the absence of Gm^{ab} in this population, the most reasonable explanations of these families are that the children are extra-marital or that the allele Gm^{xb} occurs in this population.

The Chinese, like the North and South American Indians, and the Eskimos (Steinberg *et al.*, 1961), and the Japanese (Ottensooer, 1961; Ropartz, Rivat, Rousseau, and Lenoir, 1961) appear to have the alleles Gm^{ab}, Gm^a, and Gm^{ax}. The frequencies of the alleles Gm^{ab}, Gm^a, and Gm^{ax} among the Chinese, as determined by the maximum likelihood equations published by Steinberg *et al.*

(1961), are .741, .231, and .028, respectively. The comparable frequencies among the Japanese (as determined from the combined data of Ottensooser, and of Ropartz *et al.* by the same equations) are .108, .681, and .211. It is obvious without testing, that the gene frequencies are very different in these two populations.

The agreement between the observed phenotypic frequencies in the Chinese sample and the expected frequencies computed on the basis of the above quoted gene frequencies is satisfactory ($\chi^2_{(1)} = 0.6865$; $.5 > P > .3$). The hypothesis concerning the alleles in this population gains support from the family data (table 7). The estimated frequency of ab × ab matings (computed with $Gm^{ab} = .741$ and $Gm^{a} = .231$) is .794, the observed frequency is .795 (35/44). On the basis of the estimated gene frequencies, 2.3 of the 62 children from these matings would be expected to be Gm(a); one was. Thirty-eight point four per cent of the ab individuals are expected to be Gm^{ab}/Gm^{a}, and half their offspring (19.2%) by Gm^{a}/Gm^{a} mates would be expected to be Gm^{a}/Gm^{a}. There were eight offspring from such matings, hence 1.5 would be expected to be Gm(a); one was.

SUMMARY

The ABO, MN and Rh blood types, and the Hp, Tf, and Gm [Gm(a), Gm(x), Gm(b), and Gm-like] factors were determined for 128 unrelated Indians (parents of families, 63 with two parents tested and two with one parent tested), and 90 unrelated Chinese (parents of 46 families, 44 with two parents tested and two with one parent tested), and for the offspring from these families.

The frequencies of the several blood types are presented. They were done primarily to aid in paternity testing. They compare favorably with the findings of previous studies.

The allele Hp^1 is rare in the Indian population (.09) and relatively infrequent in the Chinese (.29). Unfortunately, the data shed no light on the problem of the inheritance of the phenotype Hp O.

Only Tf C was found among the Indians. About four per cent of the Chinese were heterozygous for Tf CD_1, all other were Tf CC.

The Indians have a high frequency of Gm(a) and of Gm(x), and a low frequency of Gm(b). They appear to have alleles Gm^{a}, Gm^{ax}, and Gm^{b} in the following frequencies: .535, .234(5), and .230(5), respectively. Three families appear to have a Gm^{xb} allele, providing the offspring are not extra-marital. The Chinese appear to have the alleles Gm^{ab}, Gm^{a}, and Gm^{ax} in the following frequencies: .741, .231, and .028, respectively.

REFERENCES

GIBLETT, ELOISE R. AND A. G. STEINBERG 1960. The inheritance of serum haptoglobin types in American Negroes: Evidence for a third allele Hp^{2M}. *Am. J. Human Genet.* 12: 160–169.

KIRK, R. L., L. Y. C. LAI, SYED MAHMOOD, AND R. B. SINGH 1960. Haptoglobin types in South-East Asia. *Nature* 185: 185–186.

LAI, L. Y. C. 1961. Serum transferrins in Malays, Chinese and Indians in Malaya. *Aust. J. Sci.* 23: 228.

MOURANT, A. E. 1954. *The Distribution of the Human Blood Groups.* Springfield, Ill.: C. C Thomas.

MOURANT, A. E., ADA C. KOPEĆ, AND KAZIMIERA DOMANIEWSKA-SOBCZAK 1958. *The ABO Blood Groups*. Oxford: Blackwell, Ltd.

OTTENSOOSER, F., N. LEON, A. B. CUNHA, AND M. SATO 1961. On Gm groups in Japanese. *Lancet* (in press).

RACE, R. R. AND RUTH SANGER 1958. *Blood Groups in Man*. 3rd ed. Oxford: Blackwell, Ltd.

ROPARTZ, C., L. RIVAT, P.-Y. ROSSEAU, AND J. LENOIR 1961. Les facteurs Gma, Gmb, Gmx, Gm-like, InV chez les Japonais. *Rev. Franç. Études Clin. et Biol.* (in press).

SIMMONS, R. T., J. J. GRAYDON, N. M. SEMPLE, AND R. GREEN 1950. The A_1-A_2-B-O, M-N, and Rh blood groups in southern Chinese: Hak-Kas, Cantonese, and Hokkas. *Med. J. Aust.* ii: 917–922.

SMITHIES, O. 1959. An improved procedure for starch-gel electrophoresis: further variation in the serum proteins of normal individuals. *Biochem. J.* 71: 585–587.

STEINBERG, A. G., RACHEL STAUFFER, AND S. BOYER 1960. Evidence for a Gm^{ab} allele in the Gm system of American Negroes. *Nature* 188: 169–170.

STEINBERG, A. G., RACHEL STAUFFER, B. S. BLUMBERG, AND H. FUDENBERG 1961. Gm phenotypes and genotypes in U. S. Whites and Negroes, in American Indians and Eskimos, in Africans, and in Micronesians. *Am. J. Human Genet.* 13: 205–213.

APPENDIX

Data on the ABO, MN, Rh blood types and the haptoglobin, transferrin, and Gm serum factors for Indian and Chinese families from Malaya. The red blood cells were tested with anti-A, anti-B, anti-A+B, anti-A_1 (seed), anti-M, anti-N, anti-C, anti-D, anti-E, and anti-c. All E positive cells were tested with anti-e. For the Rh system only positive reactions are recorded. Phenotypes are recorded for the ABO and MN systems. All sera were tested for Gm(a), Gm(b), Gm(x), and Gm-like. All sera were negative for Gm-like, therefore only the reactions for the first three Gm factors are entered in the table. (M = mother, F = father, a = first child, b = second child, etc.)

CHINESE FAMILIES

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
Ch 1	M	A_1	M	CD	2-2	CC	+	+	−
	F	A_1	M	CD	2-2	CC	+	+	−
	a	A_1	M	CD	0-0	CC	+	+	−
	b	A_1	M	CD	2-2	CC	+	+	−
Ch 2	M	O	N	CDEce	2-1	CC	+	+	−
	F	O	MN	CD	2-2	CC	+	+	−
	a	O	N	CD	0-0	CC	+	+	−
	b	O	MN	CDEce	2-2	CC	+	+	−
Ch 3	M	A_1	MN	CDEce	2-2	CC	+	+	−
	F	O	MN	CD	2-1	CC	+	+	−
	e	A_1	MN	CDEce	2-1	CC	+	+	−
	g	A_1	N	CD	2-1	CC	+	+	−
Ch 4	M	O	M	CD	2-2	CC	+	−	−
	F	O	N	CD	2-1	CC	+	+	−
	b	O	MN	CD	2-2	CC	+	+	−
	c	O	MN	CD	2-2	CC	+	+	−
Ch 5	M	O	M	CD	2-2	CC	+	+	−
	F	A_1	M(N?)	CD	2-1	CC	+	+	−
Ch 6	M	B	N	CDEce	0-0	CC	+	+	−
	F	A_1	N	CDEce	2-2	CC	+	+	−
	a	B	N	CD	2-2	CC	+	+	−
	b	A_1B	N	(C?)DEc	2-2	CC	+	+	−

CHINESE FAMILIES—*Continued*

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
Ch 7	M	O	N	CDEce	2-1	CC	+	+	−
	F	B	MN	CDEce	2-1	CC	+	+	−
	a	B	N	DEc	2-1	CC	+	+	−
	b	B	N	DEc	2-1	CC	+	+	−
Ch 8	M	O	MN	CD	2-1	CC	+	+	−
	a	O	MN	CD	2-2	CC	+	+	−
Ch 10	M	B	MN	CDEe	2-1	CC	+	+	−
	F	B	M	DEc	2-2	CC	+	+	−
	a	B	MN	CDEc	2-2	CC	+	+	−
	b	B	MN	CDEc	2-1	CC	+	+	−
Ch 11	M	B	M	DEc	2-2	CC	+	+	−
	F	O	N	CD	2-1	CC	+	+	−
	c	O	MN	CDEce	2-2	CC	+	+	−
Ch 12	M	O	M	CD	2-2	CC	+	+	−
	F	O	N	CD	2-1	CC	+	+	−
	a	O	MN	CD	2-1	CC	+	+	−
	b	O	MN	CD	2-2	CC	+	+	−
Ch 13	M	A_1	M	CDEce	2-1	CC	+	+	−
	F	B	MN	CDe	2-2	CC	+	+	−
	a	O	MN	CD	2-1	CC	+	+	−
	b	B	MN	DEce	2-1	CC	+	+	−
Ch 14	M	B	MN	CDEce	2-1	CC	+	+	−
	F	O	MN	CD	2-2	CD_1	+	+	−
	a	B	M	CDEce	2-2	CD_1	+	+	−
	b	O	M	CDEce	2-2	CD_1	+	+	−
Ch 16	M	O	N	CDe	2-1	CC	+	−	−
	F	O	N	DEc	2-1	CC	+	+	−
	a	O	N	DEce	1-1	CC	+	+	−
	b	O	N	(C?)DEce	2-2	CC	+	+	−
Ch 17	M	A_1	MN	CD	2-2	CC	+	+	−
	F	O	MN	DEc	2-1	CC	+	+	−
	a	O	N	CDEce	2-1	CC	+	+	−
	b	O	MN	CDEce	2-2	CC	+	+	−
Ch 19	M	O	N	CDEce	2-2	CC	+	+	−
	F	O	MN	CD	2-2	CC	+	+	−
	a	O	N	CDEce	2-2	CC	+	+	−
	b	O	N	CD	2-2	CC	+	+	−
Ch 20	M	B	M	CD	2-1	CC	+	+	−
	F	O	N	CDEce	2-2	CD_1	+	+	−
	a	B	MN	CD	2-2	CC	+	+	−
Ch 21	M	B	N	CDEce	2-2	CC	+	+	−
	F	A_1	MN	CDEce	2-2	CC	+	+	−
	a	A_1	MN	CDEce	2-2	CC	+	+	−
	b	A_1B	MN	CD	2-2	CC	+	+	−
Ch 22	M	A_1	M	C(D?)Ece	2-1	CC	+	+	−
	F	O	MN	CDEce	2-1	CC	+	+	−
	a	A_1	MN	CDEce	2-1	CC	+	+	−
	b	A_1	MN	CDEce	2-2	CC	+	+	−
Ch 23	M	A_1	MN	CDEce	2-1	CC	+	+	−
	F	A_1B	N	CDEce	2-1	CC	+	+	−
	a	A_1	MN	CDEce	2-2	CC	+	+	−
	b	A_1B	MN	CDEce	2-1	CC	+	+	−

CHINESE FAMILIES—*Continued*

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
Ch 24	M	B	M	CD	2-1	CC	+	+	−
	F	B	MN	CD	2-2	CC	+	+	−
	b	B	M	CDEce	2-1	CC	+	+	−
	c	O	N	CD	2-2	CC	+	+	−
Ch 25	M	A_1	MN	CD	2-2	CC	+	+	−
	F	A_1	MN	(C?)DEc	1-1	CC	+	+	−
	b	A_1	M	C(D?)Ec(e?)	2-1	CD_1	+	+	−
	c	A_1	M	CDEce	2-1	CC	+	+	−
Ch 26	M	O	MN	CD	2-1	CC	+	+	−
	F	O	MN	CD	2-2	CC	+	+	−
	a	O	N	CD	2-1	CC	+	+	−
	b	O	MN	CD	2-1	CC	+	+	−
Ch 27	M	A_1	MN	CDEce	2-2	CC	+	−	+
	F	B	M	C(D?)Ece	0-0	CC	+	+	+
	a	A_1B	M	CDEce	2-2	CC	+	+	−
	b	B	M	C(D?)Ece	0-0	CC	+	−	+
Ch 28	M	B	MN	CD	2-2	CC	+	+	−
	F	A_1	M	CDEce	2-1	CC	+	+	−
	a	O	M	CD	2-2	CC	+	+	−
	b	O	M	CD	2-2	CC	+	+	−
Ch 29	M	B	N	CDEce	2-2	CC	+	+	−
	F	O	MN	CDEce	1-1	CC	+	+	−
	a	O	MN	CDEce	2-1	CC	+	−	−
	b	O	MN	DEc	2-1	CC	+	+	−
Ch 30	M	O	MN	CD	2-2	CC	+	+	−
	F	A_1B	N	DEce	1-1	CC	+	+	−
	a	A_1	MN	CDEce	2-1	CC	+	+	−
	c	A_1	MN	CDEce	2-1	CC	+	+	−
Ch 31	M	O	M	CD	2-2	CC	+	+	−
	F	O	M	CDEce	2-1	CC	+	+	−
	a	O	M	CDEce	2-1	CC	+	+	−
	b	O	M	CDEce	2-1	CC	+	+	−
Ch 32	M	A_1	MN	DEc	2-2	CC	+	+	−
	F	B	MN	CD	1-1	CC	+	+	−
	b	B	MN	CDEce	2-1	CC	+	+	−
	c	A_1	MN	C(D?)Ece	0-0	CC	+	+	−
Ch 33	M	O	M	CD	2-2	CC	+	+	−
	F	O	MN	CDc	2-1	CC	+	+	−
	a	O	M	CD	2-2	CC	+	+	−
	b	O	M	CD	0-0	CC	+	+	−
Ch 34	M	B	N	CDEce	1-1	CC	+	+	−
	F	B	N	CD	2-2	CC	+	+	−
	a	B	N	CDEce	2-1	CC	+	+	−
Ch 35	M	B	MN	CD	2-2	CC	+	−	+
	F	A_1	N	CDEce	2-2	CD_1	+	+	−
	a	A_1B	MN	CDEce	2-2	CD_1	+	+	+
	b	A_1B	MN	CDEce	2-2	CD_1	+	+	+
Ch 36	M	A_1	N	DEc	2-2	CC	+	+	−
	F	O	M	CDc	2-1	CC	+	+	−
	b	O	MN	DEce	2-2	CC	+	+	−

CHINESE FAMILIES—*Continued*

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
Ch 37	M	O	N	CDEce	0-0	CC	+	+	−
	F	O	MN	CDEce	2-2	CC	+	+	−
	a	O	N	DEc	0-0	CC	+	+	−
	b	O	N	CDEce	2-2	CC	+	+	−
Ch 38	M	A_1	MN	CD	2-1	CC	+	+	−
	F	A_1	M	C(D?)Ece	2-2	CC	+	−	−
	a	A_1	MN	CDEce	2-1	CC	+	+	−
	b	A_1	MN	CD	2-1	CC	+	−	−
Ch 39	M	O	MN	CDEce	1-1	CC	+	+	−
	F	A_1	MN	CDc	2-2	CC	+	+	−
	a	O	MN	CDc	2-1	CC	+	+	−
	b	A_1	M	CD	2-1	CC	+	+	−
Ch 40	M	O	N	CD	2-2	CC	+	+	−
	F	A_1	MN	CD	2-2	CC	+	+	−
	a	O	MN	CD	2-2	CC	+	+	−
	b	O	MN	CD	2-2	CC	+	+	−
Ch 41	M	A_1	MN	CD	1-1	CC	+	+	−
	F	O	MN	CD	2-2	CC	+	+	+
	a	A_1	M	CD	0-0	CC	+	+	−
	b	A_1	MN	CD	2-1	CC	+	+	−
Ch 42	M	B	M	CDEce	2-2	CC	+	+	−
	F	O	M	CD	2-1	CC	+	+	−
	b	O	M	CDEce	2-1	CC	+	+	−
Ch 43	M	O	M	CD	2-2	CC	+	+	−
	F	B	MN	CD	2-2	CC	+	+	+
	a	O	M	CD	2-2	CC	+	+	+
	b	B	MN	CD	2-2	CC	+	+	−
Ch 44	M	B	MN	CDEce	2-2	CC	+	+	−
	F	A_1	MN	CD	2-2	CD_1	+	+	−
	a	B	MN	CDEce	2-2	CD_1	+	+	−
	b	A_1B	MN	CDEce	2-2	CC	+	+	−
Ch 45	M	B	MN	CD	1-1	CC	+	+	−
	F	B	M	CD	2-2	CC	+	+	−
	a	B	MN	CD	2-1	CC	+	+	−
	b	B	M	CD	2-1	CC	+	+	−
	c	B	M	CD	2-1	CC	+	+	−
Ch 46	M	O	MN	DEce	2-2	CC	+	+	−
	F	O	N	DEc	2-1	CC	+	+	−
	b	O	N	DEc	2-2	CC	+	+	−
	c	O	N	DEc	2-1	CC	+	+	−
Ch 47	F	B	MN	CD	2-1	CC	+	+	−
	a	B	M	CDEce	1-1	CC	+	+	−
	b	B	M	CDEce	1-1	CC	+	+	−
Ch 48	M	B	MN	CD	2-1	CC	+	+	−
	F	B	M	CDEce	1-1	CC	+	+	−
	a	B	MN	CD	1-1	CC	+	+	−
	b	B	M	CD	1-1	CC	+	+	−
Ch 49	M	O	MN	CDEce	2-2	CC	+	+	−
	F	O	M	CDc	2-1	CC	+	−	−
	a	O	MN	CD	2-2	CC	+	+	−
	b	O	M	CDEce	2-2	CC	+	+	−

INDIAN FAMILIES

Family	Individ.	ABO	MN	Rh	HP	Tf	Gm		
							a	b	x
I 3	M	O	MN	CD	2-2	CC	+	−	+
	F	B	MN	CDEce	0-0	CC	+	−	+
	a	O	N	CDEce	2-1	CC	+	−	+
	b	O	MN	CDEce	2-1	CC	+	−	−
I 4	M	O	MN	CDEce	2-2	CC	+	−	+
	F	A_1	M	CD	2-2	CC	+	−	+
	a	O	MN	CD	2-2	CC	+	−	+
	b	B	M	CDEce	2-2	CC	+	−	−
I 5	M	O	N	CDe	2-2	CC	+	−	+
	F	A_1B	M	CD	2-2	CC	+	−	−
	a	B	MN	CD	2-2	CC	+	−	−
	b	B	MN	CD	2-2	CC	+	−	+
I 6	M	O	M	CDe	2-2	CC	+	−	−
	F	O	M	CD	2-2	CC	+	+	−
	a	O	M	CD	2-2	CC	+	+	−
	b	O	M	CDe	2-2	CC	+	+	−
I 7	M	B	M	CD	2-2	CC	+	+	−
	F	B	MN	CD	2-2	CC	+	−	−
	a	B	MN	CD	2-2	CC	+	−	−
	b	O	M	CD	2-2	CC	+	+	−
I 8	M	O	N	CDEce	2-2	CC	+	+	−
	F	A_1	M	CDe	2-2	CC	+	−	+
	a	A_1	MN	CD	2-2	CC	+	−	+
	b	A_1	MN	CDEce	2-2	CC	+	+	+
I 9	M	A_1B	M	e	2-2	CC	+	−	+
	F	A_1	MN	e	2-1	CC	+	−	+
	a	A_1	MN	e	2-2	CC	+	−	+
	b	A_1B	M	e	2-1	CC	+	−	+
I 10	M	A_1	MN	CDe	2-2	CC	+	−	+
	F	O	MN	CDe	2-1	CC	+	−	−
	a	A_1	N	e	2-2	CC	+	−	+
	b	O	MN	e	2-1	CC	+	−	+
I 11	M	O	MN	CDe	2-2	CC	+	−	−
	F	O	MN	CD	2-2	CC	+	+	−
	a	O	M	CD	2-2	CC	+	−	−
	b	A_1	M	CDe	2-2	CC	+	−	−
I 12	M	O	MN	CDe	2-1	CC	+	−	+
	F	O	M	CDe	2-2	CC	+	−	−
	a	O	MN	CDe	2-1	CC	+	−	−
	b	O	MN	CDe	2-1	CC	+	−	+
I 13	M	B	MN	CDe	2-2	CC	+	−	−
	F	A_1	M	CDe	2-2	CC	+	−	+
	a	A_1B	MN	CDe	2-2	CC	+	−	+
	b	B	MN	CD	2-2	CC	+	−	−
I 14	M	O	M	e	2-1	CC	+	−	−
	F	A_1	N	e	2-2	CC	+	−	−
	a	A_1	MN	e	2-1	CC	+	−	−
	b	A_1	MN	e	2-2	CC	+	−	−

INDIAN FAMILIES—*Continued*

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
I 15	M	A_1	MN	CD	2-2	CC	+	−	+
	F	A_1	MN	CDe	2-1	CC	+	−	+
	a	O	MN	CD	2-1	CC	+	−	+
	b	O	M	CD	2-2	CC	+	+	+
	e	A_1	MN	CD	2-1	CC	+	+	+
I 16	M	A_2	MN	CDe	2-2	CC	+	+	−
	F	O	M	CDe	2-2	CC	+	+	−
	a	A_1	M	CDe	2-2	CC	+	+	−
	c	A_1	MN	e	2-2	CC	+	+	−
I 18	M	O	MN	CD	2-2	CC	+	−	+
	F	B	MN	CDe	2-2	CC	+	+	−
	a	B	M	CDe	2-2	CC	+	+	−
	b	B	MN	CDe	2-2	CC	+	+	−
I 19	M	A_1	MN	CDe	2-2	CC	+	−	−
	F	O	MN	CD	2-1	CC	+	+	−
	a	O	N	CD	2-1	CC	+	−	−
	b	O	M	CD	2-2	CC	+	+	−
I 20	M	O	M	CD	2-2	CC	+	−	−
	F	A_1	MN	CDe	2-2	CC	+	−	+
	a	O	M	CDe	2-2	CC	+	−	−
	b	A_1	M	CD	2-2	CC	+	−	−
I 21	M	A_1	N	CD	2-2	CC	+	−	+
	b	O	MN	CD	2-2	CC	+	−	+
I 22	M	O	MN	CD	2-2	CC	+	+	+
	F	B	MN	Ce (D^u neg.)	2-1	CC	+	+	+
	a	O	M	CD	2-2	CC	+	+	+
	c	O	MN	CD	2-1	CC	+	+	+
I 23	M	O	MN	De	2-1	CC	−	+	−
	F	A_1	MN	CDe	0-0	CC	+	+	−
	a	A_1	M	CDe	2-2	CC	−	+	−
	b	A_1	MN	CDe	2-2	CC	+	+	−
I 24	M	A_1B	M	CD	2-2	CC	+	−	−
	F	O	MN	CD	2-2	CC	+	−	+
	a	A_1	M	CD	2-2	CC	+	−	+
	b	B	M	CD	2-2	CC	+	−	+
I 25	M	O	N	CD	2-2	CC	+	+	−
	F	A_1	MN	CDe	2-2	CC	+	−	+
	a	A_1	N	CDe	2-2	CC	+	+	−
	b	A_1	N	CD	2-2	CC	+	+	−
I 26	M	O	M	CDe	2-2	CC	+	−	−
	F	O	MN	CDe	2-2	CC	+	−	−
	a	O	M	CD	2-2	CC	+	−	−
	b	O	MN	CDe	2-2	CC	+	−	−
I 27	M	A_1	M	CDe	2-2	CC	+	−	+
	F	A_1	M	CDe	2-2	CC	+	−	−
	a	A_1	M	CDe	2-2	CC	+	−	+
	b	A_1	M	CDe	2-2	CC	+	−	+
I 28	M	B	MN	CD	2-2	CC	+	−	−
	F	B	MN	CD	2-2	CC	+	−	−
	a	B	MN	CD	2-2	CC	+	−	−
	b	B	N	CD	2-2	CC	+	−	−

INDIAN FAMILIES—*Continued*

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
I 29	M	O	M	CDe	2-2	CC	+	+	+
	F	B	M	CD	2-2	CC	+	+	−
	a	B	M	CD	2-2	CC	+	−	+
	b	B	M	CDe	2-2	CC	+	+	−
I 30	M	O	M	e	2-1	CC	+	−	+
	F	A_1	MN	e	2-2	CC	+	+	−
	a	A_1	MN	e	2-2	CC	+	+	−
	b	O	MN	e	2-2	CC	+	+	+
I 31	M	O	MN	CDEce	2-2	CC	+	−	−
	F	A_1B	MN	CDe	2-1	CC	+	+	−
	a	B	M(N?)	CDEce	2-2	CC	+	+	−
	b	A_1	N	CDEce	2-1	CC	+	−	−
I 32	M	B	MN	CD	2-2	CC	+	+	+
	F	B	MN	CDe	2-2	CC	+	−	+
	a	B	MN	CDe	2-2	CC	+	+	+
	b	B	MN	CDe	2-2	CC	+	+	+
I 33	M	B	MN	CD	2-2	CC	+	−	+
	F	A_1B	MN	CDe	2-2	CC	+	−	+
	a	A_1B	MN	CDe	2-2	CC	+	−	+
	b	A_1B	MN	CDe	2-2	CC	+	−	+
I 34	M	O	MN	CD	2-2	CC	+	+	−
	F	B	MN	CDe	2-2	CC	+	+	−
	a	O	M	CDe	2-2	CC	+	+	−
	b	B	MN	CDe	2-2	CC	+	+	−
I 35	M	B	N	CD	2-2	CC	+	−	−
	F	B	MN	CDEce	2-2	CC	+	−	−
	a	B	N	CDEce	2-2	CC	+	−	−
	b	B	N	CD	2-2	CC	+	−	−
I 36	M	A_1B	M	CD	2-2	CC	+	−	+
	F	A_1	M	CD	2-2	CC	+	+	+
	a	A_1	M	CD	2-2	CC	+	−	+
	b	A_1	M	CD	2-2	CC	+	+	+
I 37	M	B	M	CD	2-2	CC	+	−	+
	F	A_1	M	CD	2-1	CC	−	+	−
	a	O	M	CD	2-1	CC	+	+	−
	b	A_1	M	CD	2-1	CC	+	+	−
I 38	M	B	MN	CDe	2-2	CC	+	−	−
	F	O	N	CD	2-2	CC	+	+	−
	a	O	MN	CD	2-2	CC	+	+	−
	b	B	MN	CDe	2-2	CC	+	−	−
I 39	M	A_1	N	CD	2-1	CC	+	+	−
	F	O	M	CD	2-2	CC	+	−	−
	a	A_1	MN	CD	2-1	CC	+	−	−
	b	A_1	MN	CD	2-1	CC	+	−	−
I 40	M	B	M	CD	2-2	CC	+	+	−
	F	B	N	CD	2-2	CC	+	+	−
	a	B	MN	CD	2-2	CC	−	+	−
	b	O	MN	CD	2-2	CC	−	+	−

INDIAN FAMILIES—*Continued*

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
I 41	M	B	M	CDe	2-2	CC	+	−	−
	F	O	MN	CD	2-1	CC	+	+	+
	a	O	M	CDe	2-1	CC	+	+	−
	b	B	MN	CDe	2-2	CC	+	+	−
I 42	M	O	N	CD	2-1	CC	+	−	−
	F	O	M	CD	2-2	CC	+	+	−
	a	O	MN	CD	2-1	CC	+	−	−
	b	O	MN	CD	2-1	CC	+	−	−
I 43	M	A_1B	M	CDe	2-2	CC	+	−	+
	F	A_1	N	CD	2-1	CC	+	+	+
	a	A_1	MN	CD	2-2	CC	+	−	+
	b	A_1	MN	CD	2-2	CC	+	+	+
I 44	M	B	M	CDe	2-2	CC	+	+	+
	F	B	MN	CDe	2-2	CC	+	−	−
	a	B	M	CDe	2-2	CC	+	+	+
	b	B	M	CD	2-2	CC	+	+	+
I 45	M	A_1	M	CDe	2-1	CC	+	−	−
	F	O	MN	CD	2-2	CC	+	−	−
	a	O	MN	CD	2-1	CC	+	−	−
	b	O	MN	(C?)D	2-1	CC	+	−	−
I 46	M	O	M	DEce	2-2	CC	+	−	−
	F	B	M	CDce	2-2	CC	+	−	−
	a	O	M	CDce	2-2	CC	+	−	−
	b	O	M	CDce	2-2	CC	+	−	−
I 47	M	B	MN	CDEce	2-2	CC	+	+	−
	F	B	MN	CD	2-2	CC	+	+	−
	a	B	N	CDe	2-2	CC	+	+	−
	b	B	M	CD	2-2	CC	−	+	−
I 48	M	O	M	CDe	2-1	CC	−	+	−
	F	B	N	CD	2-2	CC	+	+	+
	a	O	MN	CDe	2-1	CC	+	+	+
	b	O	MN	CDe	2-1	CC	+	+	−
I 49	M	O	MN	CD	2-1	CC	+	+	+
	F	A_1	MN	CD	2-1	CC	+	−	+
	a	A_1	M	CD	2-1	CC	+	+	−
	b	A_1	M	CD	2-1	CC	+	−	+
I 50	M	B	N	CD	2-2	CC	+	+	+
	F	O	MN	CD	2-2	CC	+	−	+
	a	O	MN	CD	2-2	CC	+	−	+
	b	O	N	CD	2-2	CC	+	−	+
I 51	M	B	MN	CDEce	2-2	CC	+	+	+
	F	O	MN	CDEce	2-2	CC	+	+	−
	a	B	MN	CD	2-2	CC	+	+	−
	b	B	MN	CDEce	2-2	CC	+	+	+
I 52	M	B	MN	e	2-2	CC	+	+	−
	F	O	MN	CD	2-1	CC	+	+	−
	a	B	M	CDe	2-2	CC	+	−	−
	b	B	N	CDe	2-2	CC	+	+	−

INDIAN FAMILIES—*Continued*

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
I 53	M	B	MN	c	2-2	CC	+	−	−
	F	A_2	MN	CD	2-2	CC	+	−	−
	a	A_2B	MN	CDc	2-2	CC	+	−	−
	b	A_2B	MN	CDc	2-2	CC	+	−	−
I 54	M	O	MN	CDce	0-0	CC	−	+	−
	F	O	MN	Cce	2-2	CC	−	+	−
	a	O	N	(C?)Dce	2-2	CC	−	+	−
	b	O	M	CDce	2-2	CC	−	+	−
I 55	M	B	N	CDc	2-2	CC	+	+	−
	F	B	N	c	2-2	CC	+	−	−
	a	B	N	c	2-2	CC	+	+	−
	c	B	N	CDc	2-2	CC	+	+	−
I 56	F	A_1	M	CDc	2-2	CC	+	−	+
	a	O	M	CDEce	2-2	CC	+	+	−
	b	A_2	M	CDc	2-2	CC	+	+	−
I 57	M	A_1	M	CD	2-2	CC	+	+	−
	F	A_1	MN	CDc	2-2	CC	+	−	+
	a	A_1	MN	CDc	2-2	CC	+	−	+
	b	A_1	M	CDc	2-2	CC	+	+	−
I 58	M	A_1	MN	CD	2-2	CC	+	+	−
	F	A_1	MN	c	2-2	CC	+	+	+
	a	A_1	MN	CDc	2-2	CC	+	−	−
	b	A_1	MN	CDc	0-0	CC	+	−	−
I-59	M	O	MN	CDc	2-2	CC	+	−	+
	F	A_1B	M	CD	2-2	CC	+	−	−
	a	B	MN	CD	2-2	CC	+	−	−
	b	B	M	CDc	2-2	CC	+	−	+
I 60	M	O	M	CD	2-2	CC	+	−	+
	F	O	MN	CD	2-2	CC	+	−	+
	a	O	MN	CD	2-2	CC	+	−	+
	b	O	M	CD	2-2	CC	+	−	+
I 61	M	O	MN	CDc	2-2	CC	+	−	−
	F	O	M	c	2-2	CC	+	−	−
	a	O	MN	CDc	2-2	CC	+	−	−
	b	O	MN	c	2-2	CC	+	−	−
I 62	M	O	MN	DEce	2-2	CC	+	+	−
	F	B	M	CDEce	2-2	CC	−	+	−
	a	O	M	DEce	2-2	CC	−	+	−
	b	O	M	DEc	2-2	CC	+	+	−
I 63	M	O	M	CD	2-2	CC	+	−	+
	F	A_1	M	CDEce	2-2	CC	+	−	−
	a	A_1	M	CDEce	2-2	CC	+	−	+
	b	O	M	CDEce	2-2	CC	+	−	−
I 64	M	B	MN	CDEce	2-2	CC	+	−	+
	F	A_1	MN	CD	2-2	CC	+	+	+
	a	A_1B	M	CD	2-2	CC	+	+	−
	b	A_1B	MN	CD	2-2	CC	+	−	+
I 65	M	O	MN	CD	2-1	CC	+	−	−
	F	A_1	M	CDc	2-2	CC	+	−	−
	a	O	M	CDc	2-2	CC	+	−	−
	b	O	MN	CD	2-1	CC	+	−	−

INDIAN FAMILIES—*Continued*

Family	Individ.	ABO	MN	Rh	Hp	Tf	Gm		
							a	b	x
I 66	M	O	M	CDe	2-2	CC	+	+	+
	F	B	M	CDe	2-1	CC	+	−	−
	a	O	M	CDe	2-1	CC	+	−	+
	b	B	M	CD	2-1	CC	+	−	+
I 67	M	B	MN	CDe	2-2	CC	+	+	+
	F	O	MN	CDe	2-2	CC	+	−	+
	a	B	N	CD	2-2	CC	+	−	+
	b	O	N	e	2 2	CC	+	−	+
I 68	M	A_1B	N	CD	2-1	CC	+	+	+
	F	O	M	CDe	2-2	CC	+	+	−
	a	A_1	MN	CD	2-2	CC	−	+	−
	b	B	MN	CD	2-1	CC	+	+	−

Studies on the "Group Specific Component" of Human Serum. Gene Frequencies in Several Populations *

HARTWIG CLEVE AND ALEXANDER G. BEARN

The Rockefeller Institute, New York

IN 1959 HIRSCHFELD DESCRIBED several variants of a normal serum protein (Hirschfeld, 1959b). The variants are distinguishable by immunoelectrophoresis (Grabar and Williams, 1953) as indicated by their different mobilities on agar gel. The protein belongs to the α-globulin fraction. It is easily distinguishable from the haptoglobins and so far not identified with one of the already isolated and characterized proteins of this fraction (Cleve and Bearn, 1961). Hirschfeld and co-workers, who have arbitrarily called this protein the "Group Specific Component", have presented evidence that the variants are under genetical control (Hirschfeld, Jonsson, and Rasmuson, 1960).

The material presented in this paper includes:

1. Studies on families, twin-pairs, and mother-child pairs to investigate further the mode of inheritance.

2. Results of gene frequency determinations in an American white population, American and African Negroes, North American Indians (Navajos), Eskimos, and an American Chinese population.

METHODS

Immunoelectrophoresis: In this study the microtechnique of Scheidegger modified by Hirschfeld was used (Scheidegger, 1955; Hirschfeld, 1959a). This modification consists essentially of a longer separation time and the use of a barbital buffer to which calcium lactate has been added (Laurell, Laurell, and Skoog, 1956). These modifications result in a better resolution of the overcrowded α-globulin region.

Electrophoresis was carried out at pH 8.6 for 100 minutes employing a potential gradient of about 7 volts/cm. The antiserum used was the horse-antiserum against pooled normal human serum obtained from the Commercial Service of the Pasteur Institute, Paris, France (Sérum équin anti-sérum humain normal, No. 13411 and 13412, Serpasteur, Paris).

MATERIALS

Families: The sera of 31 families with 64 offspring from a U. S. white population were studied. The material included apparently healthy families, and

Received July 12, 1961.

* This work was presented in part at the Annual Meeting of the American Society of Human Genetics in Atlantic City on May 4, 1961. This work was aided by a grant from The National Foundation and supported in part by a grant from the Public Health Service (A-1542 C3).

families from patients with Wilson's disease, agammaglobulinemia, or chromosomal aberrations.

Sera from 14 families of Northern Nigeria (Habe) were obtained. Studies on the haptoglobin and transferrin inheritance in this population have been published by Barnicot, Garlick, and Roberts (1960). The material included 25 matings with 60 offspring. Two matings, found to be inconsistent in ABO transmission and inheritance of other blood group factors (Barnicot, Garlick, and Roberts, 1960), also revealed inconsistencies in transmission of Gc and were excluded.

Sera from 11 families with 43 offspring from the Navajo Indians were available. Sera of 34 twin-pairs comprising 17 monozygotic and 17 dizygotic, and 25 samples composed of serum from mothers and the cord-blood of their new born infants were studied.

Populations: 1. U. S. white population: Sera from 122 unrelated individuals collected in New York City from blood donors and patients of The Rockefeller Institute Hospital. 2. American Negro population: Sera from 144 unrelated individuals collected in New York City from blood donors and patients of The Rockefeller Institute Hospital. 3. U. S. Chinese population: Sera from 117 unrelated individuals collected in New York City from blood donors and patients of general practitioners. These individuals were of maternal and paternal Chinese ancestry; their origin could be traced to the Kwangtung province in southern China. 4. Eskimos: Sera from 67 unrelated individuals collected by The Hamilton Health Association, Hamilton, Ontario, Canada. The Eskimos were residents from different parts of Northern Canada. Sixty-two individuals were residents from Baffin Island, four from Northern Quebec and one from Prince of Wales Island. 5 and 6. Northern Nigerians (Hausa): The material has been described in a publication on the haptoglobin and transferrin inheritance in Norther Nigerians (Barnicot, Garlick, and Roberts, 1960). The samples were collected by Dr. D. F. Roberts, Dept. of Anatomy, University of Oxford, England from fifty-two villages within a radius of 40 miles south of Katsuma, Northern Nigeria. They were obtained from two ethnic groups, Fulani and Habe. Fulani sera from 100 unrelated individuals were collected at random. Habe: 54 samples of unrelated individuals were collected at random and 49 unrelated individuals were obtained from the family material. 7. Navajo Indians: The samples were collected on the reservation of Manyfarms, Arizona by the Department of Public Health, Cornell University Medical College, New York, N. Y. The material included 152 unrelated individuals and 86 individuals from families of various sizes. The families consist of either one parent and children or sibs without available parents. The offspring of matings, where both parents were recorded, have been rejected.

THE THREE PHENOTYPES OF THE GROUP SPECIFIC COMPONENT

The immunoelectrophoretic analysis of different normal human sera is illustrated in Fig. 1. The antiserum used has revealed 18 precipitin lines, indicating 18 different antigenic components. In the α-globulin fraction differences in the electrophoretic position of precipitation lines were observed. The precipitation

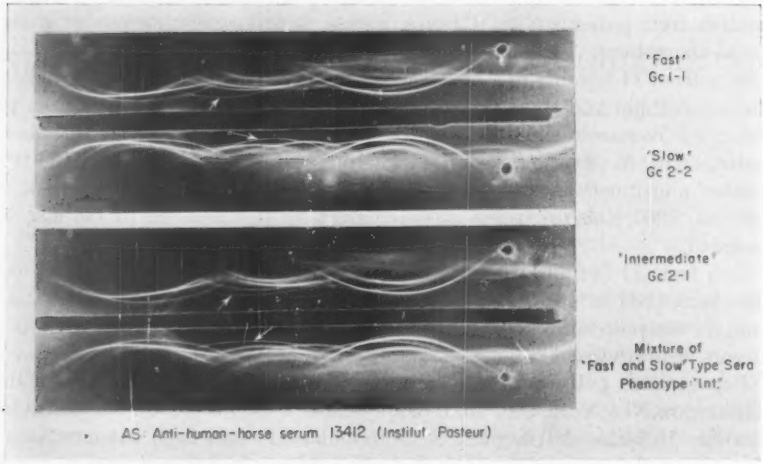

FIG. 1. A comparison of the immunoelectrophoretic patterns of the three Gc phenotypes. A comparative immunoelectrophoretic pattern of a mixture of the fast and slow phenotypes is also illustrated. The Gc is indicated by arrows. The separation has been carried out from right (cathode) to left (anode).

line of an α-globulin with three different relative electrophoretic mobilities was delineated. A fast moving component was observed in some sera (Fig. 1, first sample). In others the precipitate had the position of a slow moving component (Fig. 1, second sample). The third group of sera showed a long, rather flattened precipitation line (Fig. 1, third sample). Under optimal conditions this precipitate had two peaks, the position of the peaks corresponded to the precipitates of the fast and slow moving components. All normal human sera examined could be classified into one of these three groups. The three phenotypes may be called "Fast", "Intermediate" (or "two-peaked") and "Slow". Reproducible and clearcut variations of the "Fast" and "Intermediate" phenotypes have been observed in our laboratory and their genetical significance is under study.

The reproducibility of the classification of serum types has been investigated. Serum tested repeatedly over a nine month period and several samples of serum from the same individual, stored at $-15°C$ over a two year period, showed no alterations in phenotypes. Sera that were contaminated or stored at room temperature for a long period of time showed alterations of the immunoelectrophoretic pattern of several serum proteins. In particular, alteration of electrophoretic mobilities or the shapes of the precipitates occurred. Correct typing of the Gc groups was not possible in these instances.

STUDIES ON THE GENETIC MECHANISM

The results of the determination of the group specific component in 67 families with 167 offspring are summarized in table 1.

The family data suggest that the Gc is controlled by two co-dominant auto-

TABLE 1. GC-PHENOTYPES OF PARENTS AND CHILDREN

Parental Phenotypes	No. of Familes	Offspring			Total
		Fast	Int.	Slow	
U.S. White					
Fast-Fast	7	9	—	—	9
Fast-Int.	16	21	20	—	41
Fast-Slow	4	—	6	—	6
Int.-Int.	3	3	3	1	7
Int.-Slow	1	—	1	—	1
Slow-Slow	0	—	—	—	0
	31	33	30	1	64
Nigerian					
Fast-Fast	14	29	—	—	29
Fast-Int.	10	17	11	—	28
Int.-Int.	1	—	2	1	3
	25	46	13	1	60
Navajo					
Fast-Fast	11	43	—	—	43

somal alleles. In a fashion analogous to the notation of the haptoglobin groups, the notation Gc 1-1, Gc 2-2, and Gc 2-1 has been suggested for the serum types "Fast", "Slow", and "Intermediate", and Gc^1 and Gc^2 for the genes (Hirschfeld and Beckman, 1960).

Thirty-four twin pairs were studied; in 17 monozygotic twins the group specific component was concordant; in 17 dizygotic twins, 13 pairs had concordant types, and 4 pairs had discordant types.

Comparison of maternal and cord sera was made in 25 mother-child pairs. In 18 cases concordant Gc types were observed, in 7 cases the Gc types were discordant. In 5 cases the mother was heterozygous and the child homozygous for the Gc types in two cases the mother was homozygous and the child heterozygous. In addition to the confirmation of the suggested genetical mechanism these results indicate that the presence of the group specific component in sera of newborns is not due to placental transfer of the protein, a possibility considered because previous investigations from this laboratory had indicated that the group specific substance has a relatively slow s-rate of approximately 4S. The discordance of genotypes in mother-child pairs indicate that the fetus synthesizes its own group specific component.

The immunoelectrophoretic analysis of a mixture of equal amounts of sera from the two homozygotes reveals the phenotype characteristic of the heterozygote (Fig. 1, fourth sample).

GENE FREQUENCIES IN SEVERAL POPULATIONS

The distribution of Gc genotypes in seven populations is given in table 2. Striking differences were observed. Gene frequencies were calculated by maxi-

TABLE 2. Gc-GENOTYPES IN VARIOUS POPULATIONS

Population	Total	Gc 1-1		Gc 2-1		Gc 2-2	
		No.	%	No.	%	No.	%
Eskimo	67	34	50.75	26	38.80	7	10.45
U. S. White	122	63	51.64	49	40.16	10	8.20
U. S. Chinese	117	72	61.54	36	30.77	9	7.69
U. S. Negro	144	116	80.56	25	17.36	3	2.08
Nigerian Habe	103	88	85.44	15	14.56	—	0
Nigerian Fulani	100	90	90.00	10	10.00	—	0
Navajo	245	235	95.92	9	3.67	1	0.41

TABLE 3. ESTIMATION OF GENE RATIO FOR Gc-GENES IN A NAVAJO INDIAN POPULATION

Number in Family*	Total Indiv.	a Gc 1-1	b Gc 2-1	c Gc 2-2	b + 2a = x	2 (a + b + c) = y	Weight per Gene w
Unrelated	159	152	7	—	311	318	1.000,000
2	46	43	2	1	88	92	.666,667
3	15	15	—	—	30	30	.500,000
4	4	4	—	—	8	8	.400,000
5	15	15	—	—	30	30	.333,333
6	6	6	—	—	12	12	.285,714
	245	235	9	1	479	490	

S (wx) = 401.296 Gc^1 = 0.9765
S (wy) = 410.962 Gc^2 = 0.0235

* Family consists of either one parent and children or children alone.

mum likelihood estimates, and in the Navajo population by Cotterman's gene weighting method (Cotterman, 1947). Application of the gene weighting method to the Navajo population is demonstrated in table 3. Gene frequencies for Gc^1 and Gc^2 and standard errors (s′) of the estimates are given in table 4. The frequency for Gc^2 varies from 0.30 in Eskimos to 0.02 in Navajo Indians. Eskimos, whites, and Chinese have relative high frequencies of Gc^2, 0.30, 0.28, and 0.23, respectively. Gc^2 is lower in frequency in American Negroes (0.11). The two populations of African Negroes studied have relatively low frequencies of Gc^2, 0.07 (Habe) and 0.05 (Fulani). Gc^2 is rare in Navajo Indians, where the frequency was 0.02.

Homogeneity-χ^2-tests indicate significant differences between the gene frequencies of the white, American Negro, African Negro, and Navajo populations. The gene frequencies among the Eskimos, whites, and Chinese were not significantly different. Similarly the two populations from Northern Nigeria did not differ significantly. The frequencies in the U. S. white population differ slightly from that in a Swedish population, where the frequency of Gc^2 was 0.26 (Hirschfeld, Jonsson, and Rasmuson, 1960).

Goodness of fit of the observed and expected distribution according to the Hardy-Weinberg equilibrium has been calculated by Chi-square (table 5). Because of the relatively small size of the samples and the rareness of Gc^2 in the

TABLE 4. FREQUENCIES OF Gc ALLELES IN VARIOUS POPULATIONS

Population	Gc^1	Gc^2	s'
Eskimo	0.7015	0.2985	0.0395
U. S. White	0.7172	0.2828	0.0289
U. S. Chinese	0.7693	0.2307	0.0275
U. S. Negro	0.8924	0.1076	0.0183
Nigerian Habe	0.9272	0.0728	0.0181
Nigerian Fulani	0.9500	0.0500	0.0154
Navajo	0.9765	0.0235	0.0075

s' = standard error of the gene frequency estimates

TABLE 5. GOODNESS OF FIT OF HARDY-WEINBERG EQUILIBRIUM IN VARIOUS POPULATIONS

Population	χ^2	P
Eskimo	0.361	$>.50$
U. S. White	0.012	$>.90$
U. S. Chinese	2.080	$>.10$
U. S. Negro	1.335	$>.20$
Nigerian Habe	0.635	$>.30$
Nigerian Fulani	0.277	$>.50$
Navajo	8.577	$>.01$

two Northern Nigerian populations and the Navajo Indian population the formula developed by Levene has been applied for the calculation of the expected number of the various genotypes (Levene, 1949). The Chi-square tests for goodness of fit differ only very slightly from the figures given in table 5. In the Habe, the χ^2 was 0.587, $P > .30$; in the Fulani 0.252 and $P > .50$. In the Navajo Indians after application of Cotterman's gene weighting method and using Levene's formula a χ^2 value of 3.386 was found, $P > .05$. All the populations are in equilibrium except the Navajo Indians where the very low frequency of Gc^2 does not permit meaningful equilibrium calculations.

The observed differences in gene frequencies indicate that the group specific component will be a valuable additional marker for the characterization of populations and potentially useful in calculations on the dynamics of hybrid populations (Glass and Li, 1953; Steinberg, Stauffer, and Boyer, 1960). A reasonable calculation of the admixture of genes from white ancestors in the American Negro population should await the collection of more extensive data on gene frequencies in African Negroes.

SUMMARY

1. The three phenotypes of the group specific component of human serum have been described. The mode of inheritance as a two allelic system without dominance suggested by Hirschfeld and co-workers, was confirmed.

2. Gene frequencies in Eskimos, U. S. whites, U. S. Chinese, American Negroes, African Negroes, and Navajo Indians were reported. The frequency of Gc^2 was relatively high in Eskimos, U. S. whites, and U. S. Chinese, and was lower in American and African Negroes. Gc^2 was relatively rare in Navajo Indians.

ACKNOWLEDGMENTS

We are greatly indebted to Professor N. A. Barnicot, Department of Anthropology, University College, London for the sera and information from the Northern Nigerians, and to Dr. D. F. Roberts, Department of Human Anatomy, University of Oxford, who collected the samples. The sera from the Navajo Indians were obtained through the kindness of Drs. Walsh McDermott, Kurt Deuschle and David Rabin of the Navajo-Cornell Field Health Research Project conducted at Manyfarms, Arizona by the Department of Public Health, Cornell University Medical College, New York, New York. For the Eskimo sera, we would like to thank Dr. A. R. Armstrong and Dr. E. G. Warburton of the Hamilton Health Association, Hamilton, Ontario. We are indebted to Dr. Richard H. Osborne, of the Sloan-Kettering Institute, and to the Damon Runyon Grant #518, for sera and information on twin pairs. The collection of sera from U. S. Chinese was made possible through the kind assistance given us by Drs. W. Ling, S. Meltzer, and R. Quan.

REFERENCES

Barnicot, N. A., Garlick, J. P., and Roberts, D. F. 1960. Haptoglobin and transferrin inheritance in Northern Nigerians. *Ann. Human Genet.* 24: 171–183.

Cleve, H., and Bearn, A. G. 1961. Inherited variations in human serum proteins: Studies on the group specific component. *Ann. N. Y. Acad. Sci.* 94: 218–224.

Cotterman, C. W. 1947. A weighting system for the estimation of gene frequencies from family records. *Contrib. Laboratory of Vertebrate Biology, Univ. of Michigan.* 33: 1–21.

Glass, B., and Li, C. C. 1953. The dynamics of racial intermixture—an analysis based on the American Negro. *Am. J. Human Genet.* 5: 1–20.

Grabar, P. and Williams, C. A. 1953. Méthode permettant l'étude congugée des propriétés électrophorétiques et immunochimiques d'un mélange de proteines. Application au sérum sanguin. *Biochim. Biophys. Acta* 10: 193–194.

Hirschfeld, J. 1959a. Individual precipitation patterns of normal rabbit sera. *Acta Pathol. Microbiol. Scand.* 46: 229–238.

Hirschfeld, J. 1959b. Immune-electrophoretic demonstration of qualitative differences in human sera and their relation to the haptoglobins. *Acta Pathol. Microbiol. Scand.* 47: 160–168.

Hirschfeld, J., Jonsson, B., and Rasmuson, M. 1960. Inheritance of a new group specific system demonstrated in normal human sera by means of an immunoelectrophoretic technique. *Nature* 185: 931–932.

Hirschfeld, J., and Beckman, L. 1960. A new group-specific serum system (Gc-groups) in relation to blood and serum groups. *Acta Genet. Stat. Med.* 10: 48–53.

Laurell, C. B., Laurell, S., and Skoog, N. 1956. Buffer composition in paper electrophoresis; considerations on its influence, with special reference to the interaction between small ions and proteins. *Clinical Chem., N. Y.* 2: 99–111.

Levene, H. 1949. On a matching problem arising in genetics. *Ann. Math. Stat.* 20: 91–94.

Scheidegger, J. J. 1955. Une micro-méthode de l'immunoélectrophorèse. *Intern. Arch. Allergy.* 7: 103–110.

Steinberg, A. G., Stauffer, R., and Boyer, S. 1960. Evidence for a Gm^{ab} allele in the Gm system of American Negroes. *Nature* 188: 169–170.

A Genetic Study of Hereditary Renal Dysfunction with Associated Nerve Deafness

MAIMON M. COHEN*, GEORGE CASSADY†, AND BERTRAM L. HANNA*

IN RECENT YEARS the syndrome of hereditary renal dysfunction and associated nerve deafness has been studied by a number of investigators. Some clinical components of this syndrome have been described, but its mode of inheritance has remained in question.

Twenty-two families with this disorder have been cited in the literature to date. Although reports in the late 1800's were suggestive of this syndrome, Guthrie (1902) was the first to thoroughly study and document a family. This family was further studied at Guy's Hospital over a twenty-five year period (Kendall and Hertz, 1912; Hurst, 1923; Alport, 1927). Nineteen of the twenty-nine members in three generations of this family were affected with various stigmata of the disease. Nine exhibited renal involvement and deafness, eight renal involvement alone and two showed deafness unassociated with renal disease. The various authors concluded that the syndrome is transmitted as a dominant trait.

Perkoff *et al.* (1951, 1958) studied extensively a large family containing 217 individuals of which 205 were examined (see also Stephens *et al.* 1951). These data were interpreted as compatible with partially sex-linked dominant inheritance with $15.4 \pm 5.8\%$ crossover. Deafness was found almost exclusively in males. Perkoff (1960, 1961) reported a second family with 152 members with a clinically similar syndrome, but the mode of inheritance appears to be autosomal dominant.

Other investigators have reported small families that suggest a partially sex-linked mode of inheritance, but in these cases, the absence of offspring from affected males makes this conclusion questionable. Poli (1955) reported a family of 92 members of which 28 were examined and 17 found to be affected. The syndrome is stated to be an autosomal dominant.

Re-evaluation of some of the reported cases led Graham (1959, 1960) to conclude that this disease is an autosomal dominant, and that the apparent sex deviation results from excessive early mortality in males.

Shaw and Glover (1961) have proposed that the trait is inherited as an auto-

Received June 21, 1961.

* Human Genetics Section, Clinical Investigations Branch, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda 14, Maryland.

† Medical Investigations Section, Clinical Investigations Branch, National Institute of Dental Research, National Institutes of Health, Public Health Service, U.S. Department of Health, Education and Welfare, Bethesda 14, Maryland.

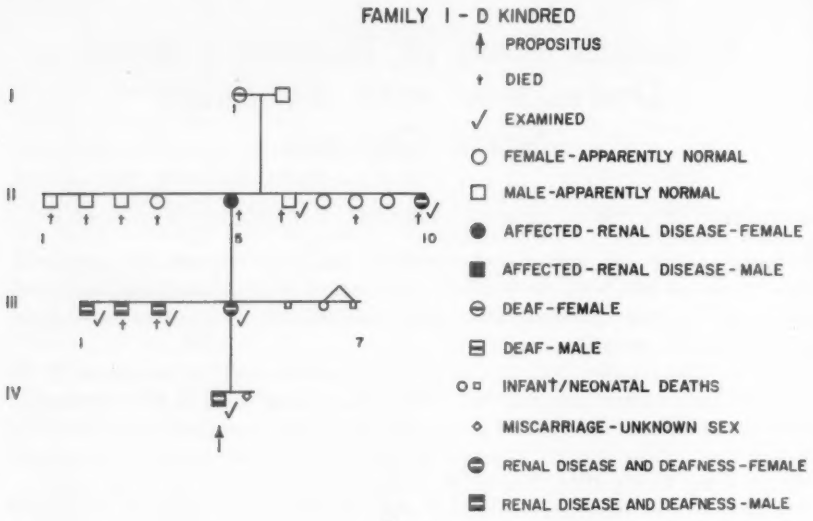

FIG. 1.

somal dominant with non-random segregation and preferential association of the autosome carrying the gene with the X chromosome. The present study provides additional support for this hypothesis and also provides the first data for conclusively excluding other postulated modes of inheritance.

This study of five previously unreported families was initiated in 1957 at the Duke Hospital Pediatric Renal Clinic when the first of four unrelated children was found with chronic nephritis. In the past year an additional family, ascertained through an unrelated adult propositus at the National Institutes of Health, was added. The detailed clinical and laboratory studies carried out on the members of these families will be reported elsewhere. (Cassady *et al.*, in preparation).

Three of the five families studied are located in North Carolina (Figs. 2, 3, 4), one is in Virginia (Fig. 5) and one is widely distributed across the United States (Fig. 1). One family migrated to this country from Switzerland two generations ago (Fig. 1) and one is of German descent but has resided for six generations in the United States (Fig. 5). The remaining three families are primarily of Anglo-Saxon extraction.

Physical examinations and extensive histories were taken of all available members in each of the five families. A total of 281 individuals were examined, of whom 147 were affected. Urine and blood samples were obtained from nearly all living individuals. Urine was centrifuged and the sediment analyzed within two hours after collection. All medical and laboratory procedures were performed by a single physician (G.C.). Blood was immediately placed in A.C.D. solution and refrigerated for blood typing. Audiograms were obtained from 86 individuals, 26 of whom show a specific audiometric defect. Clinical evidence of

FAMILY 2 - M KINDRED

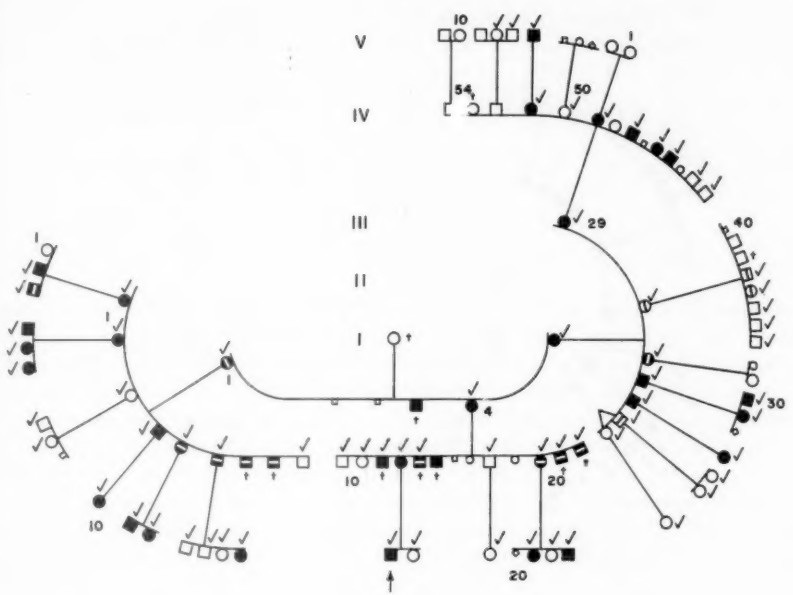

FIG. 2.

FAMILY 3 - H KINDRED

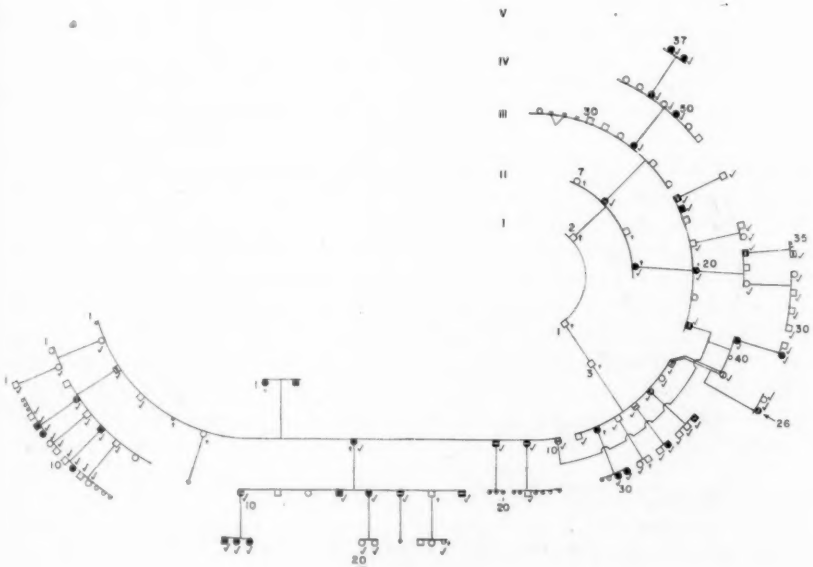

FIG. 3.

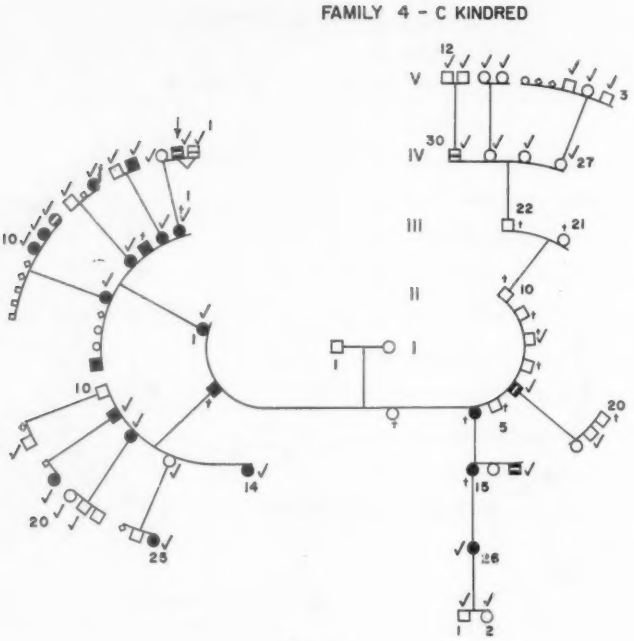
FAMILY 4 - C KINDRED
V
IV
III
II
I

FIG. 4.

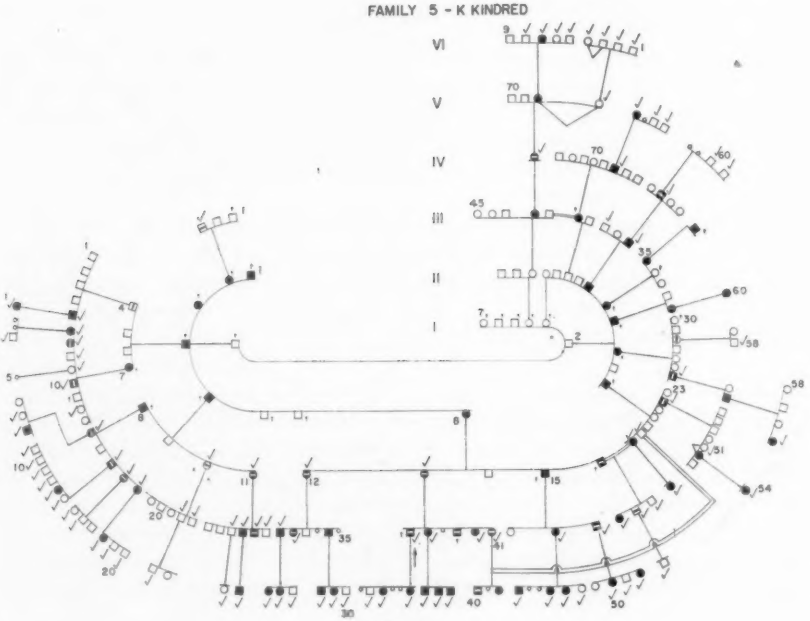
FAMILY 5 - K KINDRED
VI
V
IV
III
II
I

FIG. 5.

TABLE 1. CLASSIFICATION OF DIAGNOSED INDIVIDUALS

	Male	Female	Total
Renal disease & deafness	31	24	55
Renal disease	51	92	143
Deafness	5	2	7
Total affected	87	118	205
Normal	85	49	134
Total	172	167	339

deafness was obtained for 37 individuals. Deafness in the absence of renal disease occurs in seven affected individuals. Fifty-eight additional individuals were classified as affected, 13 on the basis of adequate physicians or hospital records or both, and forty-five by family history or pedigree construction (having a parent and offspring undoubtedly affected). Table 1 shows the classification of individuals in these families. It is seen that the frequency of deafness in males (20.9%) is slightly greater than in females (15.6%).

The diagnosis of affected and normal individuals may involve more than just the investigator's talent and experience. The intermittency of the illness poses problems in classification which can be solved only by repeated examination. This may be noted in the studies of several investigators. Guthrie (1902) examined one patient periodically for several months. Definite diagnosis was delayed until the typical atack of gross hematuria took place. The intermittent nature of the syndrome may also be seen in the data of the follow-up study of Perkoff *et al.* (1958). A total of 97 individuals were re-examined: 13 of these (13.4%) showed urinary findings sufficiently different to lead to diagnostic reclassification. These findings, as well as our clinical experience, indicate that a single examination and urinalysis is insufficient to classify individuals who are not obviously affected.

In this study, 22 individuals were re-examined because of inconclusive initial diagnoses. Fourteen of these showed urinary abnormalities on at least one re-examination; the remaining eight were classified as normal. At the time of re-examination, all available family members were also retested, comprising a group of 71 previously examined individuals (38 normal and 33 affected). Thirteen individuals initially diagnosed as normal showed urinary abnormalities on one or more follow-up examinations. Thus, of the 93 individuals repeated, 27 (29%) showed sufficient urinary changes to warrant reclassification as affected. Individuals were considered affected when they showed: More than three R.B.C. per high power field or more than five W.B.C. per high power field in fresh, centrifuged urinary sediment, or nerve deafness alone (when both a parent and child showed definite evidence of renal disease).

The severity of this syndrome encompasses a wide clinical spectrum in both sexes, ranging from an "asymptomatic" individual to the ten-year-old girl, the product of the consanguineous marriage of two mildly affected individuals, with terminal uremia and severe bilateral nerve deafness. It has been stated

repeatedly that the illness is more severe in males, usually leading to death in early life. Although this is generally the case in these families, there are several asymptomatic males (having an affected parent and offspring) and a number of severely affected females whose deaths were due to the syndrome. In the affected members of these families, 27 deaths were attributable to hereditary renal dysfunction (19 males, average age at death 23 years, 10 deaf; 8 females, average age at death 30 years, none deaf).

It is interesting to note that the severity of the affliction seems to differ among sibships in large families. In family 2 (Fig. 2), two affected sisters (II-1 & II-5) married apparently normal brothers from a family having a relatively high incidence of clinically typical sporadic acute glomerulonephritis. Offspring from these matings were very severely affected, in contrast to the mildly affected offspring of a third affected sister (II-6). Similar observations were made in family 5, where marriages with members of families with sporadic glomerulonephritis also occurred.

The proportion of affected individuals in previously reported families may be seen to be directly related to the total number of individuals examined. In the study of Guthrie's family, each successive paper dealth with a separate branch of the kindred with little or no overlap. When viewed individually, these reports show no deviation from the distribution expected if the trait is dominant. However, upon combining the results of these several studies, 66.5 per cent of the individuals are found to be affected. Poli's family has an incidence of 60.7 per cent affected among the individuals examined. Goldbloom *et al.* (1957) and Sohar (1954) have shown that nearly every child is affected in the sibships of their propositi. When pedigree construction or family history is used as a mainstay of diagnosis, it is evident, by the intermittent nature of the disease itself, that many affected family members may be called normal. The bias in the classification of individuals without complete urine examination, and re-examination where necessary, will always be toward underdiagnosis.

Preliminary linkage studies utilizing the paired sib method have revealed no linkage between this syndrome and the ABO, Rh, P, Kell and Lewis blood types or the ability to taste PTC.

Suggested Modes of Inheritance

I. Partial Sex-Linked Dominant: Most previous investigators have postulated that this syndrome is inherited as a partially sex-linked dominant trait. The absence of offspring from affected males in many of these families has made it impossible to validate this hypothesis. In the present study, affected males have reproduced and the segregation of the trait in their offspring has been used to test the hypothesis of partial sex linkage. Table 2 indicates the segregation of the trait in 12 sibships of two or more from affected fathers.

If the male carries the gene on his X chromosome, receiving it from his female parent, it would then be expected that an excess of his daughters and few of his sons would be affected. These data indicate 4 individuals (1 girl and 3 boys) who could occur only following crossover between the X and Y chromosomes, giving a crossover value of 20 percent. If, however, the male receives the

TABLE 2. CLASSIFICATION OF OFFSPRING OF AFFECTED FATHERS
TEST FOR PARTIAL SEX LINKAGE

Sex of children	Status of children	Grandfather affected	Grandmother affected	Total	Probability under partial sex linkage
Same as Father's affected parent	Affected	4	9	13	$(1 - c)/2$
	Normal	9	1	10	$c/2$
Opposite	Affected	9	3	12	$c/2$
	Normal	3	7	10	$(1 - c)/2$

Proportion of crossover (c) = 0.4889 ± 0.0236

TABLE 3. DISTRIBUTION OF OFFSPRING OF AFFECTED × NORMAL MATINGS
TEST FOR AUTOSOMAL DOMINANCE

Affected Parent	No. of Families	Offspring				$\chi^2_{(1)}$
		Male		Female		
		Affected	Normal	Affected	Normal	
Female	47	51	19	47	16	29.883**
Male	26	13	17	21	4	12.093**
Total	73	64	36	68	20	34.023**

** Denotes significance at the 0.01 level.

gene on his Y chromosome, from his father, one would expect an excess of affected sons and normal daughters. The observed distribution fails to conform to this expectation. Combining the data of both types of progeny, the crossover percentage is estimated as $48.89 \pm 2.36\%$.

The sequential method for the detection of linkage (Morton, 1957) was used to test the partial sex linkage hypothesis in these critical sibships, i.e., offspring of affected fathers. Tests made assuming both maximum and minimum crossover values ($\theta = 0.05$; $\theta = 0.40$) gave no evidence that the crossover value is significantly less than 50 per cent [$\sum$ (w-z) = -15.59 and -0.47 respectively]. Therefore it may be concluded that these data provide no support for partial sex linkage as the mode of inheritance of this syndrome.

II. Autosomal Dominant: The hypothesis of autosomal dominant inheritance was tested by analysis of the progeny of affected by normal matings. A 1:1 ratio of normal to affected is expected, but this ratio is not realized (table 3). From a re-evaluation of the data of Stephens *et al.* (1951), Graham (1959) has suggested that an apparent abnormal sex ratio exists. The deficiency of affected males is attributed to early intrauterine loss and unreported frequent abortions, miscarriages, and neonatal deaths. Perkoff *et al.* (1960) deny that this is a factor in their study. There is no significant deviation from the expected sex ratio within sibships in our families; the progeny distributions do not, however, follow that expected for an autosomal dominant trait. Affected females produce an excessive number of affected individuals of both sexes. Affected males produce a great excess of affected daughters. Among sons from affected fathers, however, the ratio approximates the classical 1:1. Tests of these two distributions indicate that these data fail to support the hypothesis of simple dominance.

III. The Role of Intra-Uterine Infection: Poli (1955) suggested that a spe-

TABLE 4. TEST OF ESTIMATED ASSOCIATION OF AUTOSOME WITH X CHROMOSOME (69.1%) USING OFFSPRING OF AFFECTED MALES

	Males		Females		$\chi^2_{(3)}$
	Affected	Normal	Affected	Normal	
Present data					
Observed	13	17	21	4	
Expected	9.27	20.73	17.27	7.73	4.76
Combined data of Perkoff *et al.*, and Shaw and Glover					
Observed	7	18	25	8	
Expected	7.73	17.27	22.80	10.20	0.79

(0.05) $\chi^2_{(3)} = 7.82$

cific nephrotoxin transplacentally transmitted may be implicated in the etiology of this inherited condition. This hypothesis may be true if the syndrome is inherited as an autosomal dominant trait which is wide-spread in the population and the specific nephrotoxic agent is rare. The excess of affected offspring from affected females is explained by this hypothesis, but not the distribution of the progeny of affected males.

IV. Preferential Segregation and Chromosomal Association: Shaw and Glover (1961) have suggested that the aberrant ratios observed in this syndrome may result from preferential segregation of chromosomes during meiosis, the chromosome bearing the defective gene being selected to pass into the functional gamete in association with the X-chromosome. The segregation ratio observed among the offspring of affected females in these families suggests that the autosome bearing the defective gene may pass to the primary oocyte 74.4 ± 3.8 per cent of the time. This frequency differs significantly from that estimated from the combined data of Perkoff *et al.*, and Shaw and Glover (55.1 ± 3.4%). Differences in the method of diagnosis employed in the three studies may account for this observed difference in frequency. Perkoff *et al.* observed a deficiency of affected males among the offspring of affected fathers, but this deficiency is not observed in the present study or in that of Shaw and Glover.

From the total offspring of affected males in the present study the frequency of association with the X-chromosome is estimated as 69.1 ± 6.2 per cent. The expected numbers of offspring in the various classes were calculated using sex ratios as observed both for our data, and for the combined data of Perkoff *et al.*, and Shaw and Glover (table 4). The two bodies of data produced $\chi^2_{(3)}$ values of 4.760 ($P > 0.10$) and 0.786 ($P > 0.80$), respectively.

DISCUSSION

The preferential passage of chromosomes to a specific spindle pole and the association of non-homologues during meiosis are known to occur in several organisms. Rhoades (1942, 1952) has shown in maize that chromosome 10 segregates preferentially during megasporogenesis when it carries on the end of the long arm an extra piece of chromatin lacking in the normal homologue. When an abnormal chromosome 10 is present, more than 70 per cent of the ovules receive

this chromosome rather than the expected 50 per cent. Longley (1945) observed preferential segregation for other chromosome pairs in the presence of an abnormal chromosome 10. This occurs, however, only in chromosome pairs known to carry a terminal satellite. Using marker genes on chromosomes number 6 and 9, with both knobbed and knobless strains of maize, Longley was able to demonstrate that in the presence of an abnormal chromosome 10, the chromosomes bearing the satellites appear in 64–69 per cent of the functional ovules. When a normal tenth chromosome is present, preferential segregation also occurs but to a lesser degree. It can therefore be stated that, in maize, not only does a single chromosome exhibit preferential segregation and selection, but certain groups of non-homologous chromosomes are associated consistently within a functional gamete.

The experiments of Sturtevant (1936) with triplo-IV in *Drosophila melanogaster* show that segregation of the three number 4 chromosomes is not random. Certain of the chromosomes pass more frequently to the same pole than would be expected under random assortment. Beadle (1935) observed a correlated orientation of non-homologous chromosomes on the equatorial plate in triploid Drosophila with attached X-chromosomes. Mather (1939) mentions that in female Drosophila heterozygous for the scute (sc^8) deficiency, the chromosome bearing this aberration was recovered twice as frequently among the daughters as was the normal homologue. In Sciara there is evidence for preferential selection of the entire maternal complement of chromosomes, as the paternal set moves away from the monocentric spindle of the primary spermatocyte (Metz, 1938).

The work of Seiler (1921) indicates the importance of environment in preferential segregation. In the moth *Talaeporia tubulosa*, during meiosis at normal and subnormal temperatures, the X-chromosome most frequently passes into the polar body. At high temperatures and in overripe eggs the X is more often left in the egg. In an analysis of the spore arrangement in the asci of the fungus *Bombardia lunata*, Catcheside (1944) found that certain genes are preferentially segregated.

Ferguson-Smith and Handmaker (1961) have recently demonstrated mitotic association among human chromosomes bearing satellites. This finding was present in 60 per cent of the mitoses studied. Constant associations of 2, 3, 4 and even 6 satellited chromosomes have been observed. If similar associations should occur during the meiotic process, preferential selection or segregation of groups rather than individual chromosomes may occur in man.

The chromosomal associations and the specific orientation of chromosomal groups on the meiotic spindle seen in these diverse living forms provide a model which will explain the aberrant ratios observed in families with hereditary renal dysfunction. There appears to be little question that the trait is inherited as an autosomal dominant. The chromosome in which the defective gene resides may be preferentially selected in association with the X-chromosome. During oogenesis in affected females, the autosome bearing the mutant gene may generally pass into the primary oocyte rather than into the polar body. The defective gene

would, more often than not, be incorporated in the functional ovum which would lead to an excess of affected offspring of both sexes. During spermatogenesis in affected males, the association of the X-chromosome and the trait bearing autosome would cause the two chromosomes to move together preferentially, but at random to either pole, leading to an excess of affected daughters and normal sons.

SUMMARY

The inheritance of hereditary renal dysfunction with associated nerve deafness has been examined in five previously unreported families in which a total of 339 members of four generations were classified on the basis of clinical examinatinons and/or reliable clinical records. The syndrome in these unrelated families is not inherited as a partially sex-linked dominant or as an autosomal dominant trait giving classical Mendelian segregation ratios, as suggested by previous workers. Evidence from experimental organisms is cited in support of the hypothesis of Shaw and Glover (1961) that the segregation ratios observed in this syndrome result from non-random chromosome segregation at the first meiotic division of gametogenesis, together with preferential association of the gene bearing autosome with the X-chromosome. Estimates obtained from these families are employed to test this hypothesis with data from the literature.

ACKNOWLEDGMENTS

We wish to thank Dr. Jarvis E. Seegmiller, NIAMD, NIH for referring his patient for study, the Duke Hospital ENT Clinic for performing audiometric examinations on a number of individuals, and Mr. Webster C. Leyshon, Medical Investigations Section, NIDR, NIH for the determination of blood group genotypes. We are indebted to Drs. John B. Graham, F. Clarke Fraser, Carl J. Witkop and Herbert L. Cooper for helpful discussion during the course of this work.

REFERENCES

ALPORT, A. C. 1927. Hereditary familial congenital haemorrhagic nephritis. *Brit. Med. J.* 1: 504–506.

BEADLE, G. W. 1935. Crossing over near the spindle attachment of the X chromosomes in attached X triploids of *Drosophila melanogaster*. *Genetics* 20: 179–191.

CASSIDY, G. E., COHEN, M. M., AND DEMARIA, W. The syndrome of hereditary renal dysfunction with associated anomalies (in preparation).

CATCHESIDE, D. G. 1944. Polarized segregation in an ascomycete. *Ann. Bot.* 8: 119–130.

FERGUSON-SMITH, M. A. AND HANDMAKER, S. D. 1961. Observations on the satellited human chromosomes. *Lancet* i: 638–640.

GOLDBLOOM, R. B., FRASER, F. C., WAUGH, D., ARONOVITCH, M., AND WIGGLESWORTH, F. W. 1957. Hereditary renal disease associated with nerve deafness and ocular lesions. *Pediatrics* 20: 241–252.

GRAHAM, J. B. 1959. Hereditary chronic kidney disease: An alternative to partial sex-linkage in the Utah kindred. *Am. J. Human Genet.* 11: 333–338.

GRAHAM, J. B. 1960. Chronic hereditary nephritis: Not shown to be partially sex-linked. *Am. J. Human Genet.* 12: 382–384.

GUTHRIE, L. G. 1902. "Idiopathic" or congenital, hereditary and family haematuria. *Lancet* i: 1243–1246.

HURST, A. F. 1923. Hereditary familial congenital nephritis occurring in sixteen individuals in three generations. *Guy's Hospital Rept.* **73**: 368–370.

KENDALL, G. AND HERTZ, A. F. 1912. Hereditary familiar congenital haemorrhagic nephritis. *Guy's Hospital Rept.* 66: 137–141.

LONGLEY, A. E. 1945. Abnormal segregation during megasporogenesis in maize. *Genetics* 30: 100–113.

MATHER, K. 1939. Crossing over and heterochromatin in the X chromosome of *Drosophila melanogaster*. *Genetics* 24: 413–435.

METZ, C. W. 1938. Chromosome behavior, inheritance and sex determination in Sciara. *Am. Nat.* 72: 485–520.

MORTON, N. E. 1957. Further scoring types in sequential linkage tests, with a critical review of autosomal and partial sex linkage in man. *Am. J. Human Genet.* 9: 55–75.

PERKOFF, G. T. 1960. Personal communication.

PERKOFF, G. T. 1961. Hereditary chronic nephritis. *In* E. L. Quinn [ed.] *Henry Ford Hospital International Symposium on the Biology of Pyelonephritis.* 259–267. Boston: Little, Brown and Co.

PERKOFF, G. T., NUGENT, C. A., JR., DOLOWITZ, D. A., STEPHENS, F. E., CARNES, W. H., AND TYLER, F. H. 1958. A follow-up study of hereditary chronic nephritis. *A.M.A. Arch. Int. Med.* 102: 733–746.

PERKOFF, G. T., STEPHENS, F. E., DOLOWITZ, D. A. AND TYLER, F. H. 1951. A clinical study of hereditary interstitial pyelonephritis. *A.M.A. Arch. Int. Med.* 88: 191–200.

PERKOFF, G. T., STEPHENS, F. E. AND TYLER, F. H. 1960. Chronic hereditary nephritis and Y chromosome linkage: Reply to Graham. *Am. J. Human Genet.* 12: 381–382.

POLI, M. 1955. Néphropathie médicale bilatérale familiae à évolution chronique. *Helv. Medica Acta* 22: 109–122.

RHOADES, M. M. 1942. Preferential segregation in maize. *Genetics* 27: 395–407.

RHOADES, M. M. 1952. Preferential segregation in maize. *Heterosis.* Ed. J. Gowen, Iowa State College Press. 66–80.

SEILER, J. 1921. Geschlechtschromosomenuntersuchungen an psychiden. I. Experimentelle beeinflussung der geschlechtsbestimmenden reifeteilung bei *Talaeporia tubulosa retz. Arch. Zellforsch.* 15: 249–268.

SHAW, R. F. AND GLOVER, R. A. 1961. Abnormal segregation in hereditary renal disease with deafness. *Am. J. Human Genet.* 13: 89–97.

SOHAR, E. 1954. A heredo-familial syndrome characterized by renal disease, inner ear deafness & ocular changes. *Harefuah* 47: 161.

STEPHENS, F. E., PERKOFF, G. T., DOLOWITZ, D. A., AND TYLER, F. H. 1951. Partially sex-linked dominant inheritance of interstitial pyelonephritis. *Am. J. Human Genet.* 3: 303–313.

STURTEVANT, A. H. 1936. Preferential segregation in triplo-IV females of *Drosophila melanogaster*. *Genetics* 21: 444–466.

Genetic Study of a Family Possessing Hemoglobins S and C, and Classical Thalassemia*

PAUL R. MCCURDY[1] AND HOWARD A. PEARSON[2]

Washington, D.C.

INTRODUCTION

DESPITE MAJOR ADVANCES in the delineation of the molecular structure of hemoglobin which have permitted a more precise understanding of the biochemical genetics of the abnormal hemoglobin syndromes (Ingram, 1961), investigation of "certain critical marriages" is indispensable for establishing the relationships of genes responsible for the hemoglobinopathies (Zuelzer, Neel, and Robinson, 1956). The clinical genetic evidence for allelism of the genes governing the synthesis of the abnormal hemoglobins S and C is based on few published pedigrees (Ranney, 1954; Smith & Conley, 1956; Neel, 1958; Smith & Krevans, 1959). Their uncertain genetic relationship to thalassemia has been partially clarified by the growing body of evidence that "thalassemia" is a broad generic term encompassing a number of distinguishable hereditary microcytoses. One of the most clearly defined varieties of the thalassemia syndrome has been designated "classical thalassemia" (Gerald and Diamond, 1958). This is an hereditary microcytosis characterized in the heterozygous state by an increase in the normal minor hemoglobin component, hemoglobin A_2.

Recently it has been possible to study a large Negro family in whose members hemoglobin S, hemoglobin C, and "classical thalassemia" occurred alone and in combination. The observed pattern of inheritance of these three entities supports the current hypothesis that the three causative genes are allelic or very closely linked.

MATERIALS AND METHODS

The data in this report are derived from the study of a family observed on the Georgetown Medical Service of the D.C. General Hospital. Quantitation of the various hemoglobin fractions was performed using starch block electropheresis in veronal buffer pH 8.6 (Pearson and McFarland, 1959). Hemoglobin F was

Received June 28, 1961.

* Supported in part by a grant, A-2823, from the National Institute of Arthritis and Metabolism, Bethesda, Maryland.

[1] Assistant Professor of Medicine, Georgetown University School of Medicine, and Medical Officer, D.C. General Hospital, Washington, D.C.

[2] Assistant Chief, Pediatric Service, U. S. Naval Hospital, Bethesda, Maryland, and Clinical Instructor in Pediatrics, Georgetown University School of Medicine.

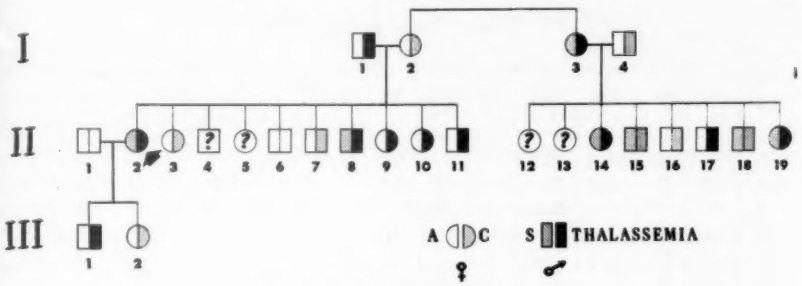

FIG. 1. Pedigree on family W.

determined by a minor modification of an alkali denaturation technique (Singer, Chernoff and Singer, 1951).

When possible, blood groupings were determined to confirm stated family relations (We are grateful to Dr. Paul Schmidt, National Institutes of Health, Bethesda, Maryland, for performing these studies.), and in no instance was the stated relationship disproved. The conventions of nomenclature suggested at a recent international conference (Nomenclature of Abnormal Hemoglobins, 1960) are used throughout this report.

RESULTS

The pedigree of the family reported in this study is illustrated in Fig. 1. Hematologic, electrophoretic and clinical data are listed in Table 1. The proband (II-2) is a 32 year old Negro female with a moderately severe hemolytic anemia. (Clinical and erythrokinetic data of this case will be reported in greater detail elsewhere.) Her red cells were characterized by marked leptocytosis, hypochromia, and poikilocytosis. Paper and starch block electrophoresis of her hemoglobin showed a C + A pattern with marked predominance of hemoglobin C.

The father of the proband (I-1) had minimal hypochromic microcytosis and an elevated proportion of hemoglobin A_2—thus fulfilling the criteria for "classical thalassemia" trait (Gerald and Diamond, 1958). The mother (I-2) had uncomplicated hemoglobin C trait.

The proband was married to a hematologically normal man (II-1). One of her children (III-1) had thalassemia trait; the other (III-2), hemoglobin C trait.

A maternal aunt (I-3) was also found to have C-thalassemia and had married an individual with sickle cell trait (I-4). The children from this union had C-trait, thalassemia trait, hemoglobin C-S disease or S-thalassemia disease.

DISCUSSION

Itano (1953) suggested that the hemoglobin S and C genes were allelic. This hypothesis was based upon the absence of hemoglobin A in the S-C doubly heterozygous individual. It has been confirmed both by pedigree analysis (Ranney, 1954; Smith & Conley, 1956; Neel, 1958; and Smith & Krevans,

TABLE 1: LABORATORY DATA

Generation No.	Age	Sex	Hct.	%A*	%S	%C	%F†	%A_2	Hb. Phenotype	Blood group							
										ABO	M	N	S	C	D	E	c	e
I_1	56	M	37.5	95.7	—	—	0.6	4.3	A-thal	O	−	+	−	−	+	−	+	+
I_2	50	F	37.5	67	—	33	0.9	—	A-C	B	−	+	+	−	+	−	+	+
I_3	38	F	39	22	—	78	6.0	—	C-thal	B	−	+	+	−	+	−	+	+
I_4	—	M	—	63	34	—	0.4	3.0	A-S	O	+	+	−	−	+	−	+	+
II_1	—	M	—	97.2	—	—	—	2.8	AA	A_1	+	+	+	−	+	−	+	+
II_2	32	F	27	28	—	72	6.8	—	C-thal	B	−	+	−	−	+	−	+	+
II_3	30	F	—	—	—	—	—	—	AC									
II_6	19	M	45	100	—	—	—	—	AA‡									
II_7	15	M	40	62	—	38	1.1	—	AC									
II_8	13	M	36	36	—	64	9.6	—	C-thal									
II_9	12	F	39	94.4	—	—	0.5	5.6	A-thal									
II_{10}	11	F	—	94.5	—	—	0.7	5.5	A-thal									
II_{11}	7	M	40	96.1	—	—	0.5	3.9	A-thal									
II_{14}	14	F	—	30	70	—	7.0	—	S-thal	O	−	+	−	−	+	−	+	+
II_{15}	12	M	—	—	40	60	1.1	—	CS	O	−	+	+	−	+	−	+	+
II_{16}	10	M	—	62	—	38	0.3	—	AC	B	−	+	+	−	+	−	+	+
II_{17}	8	M	—	94.9	—	—	1.7	5.1	A-thal									
II_{18}	7	M	—	—	43	57	0.2	—	CS	O	−	+	+	−	+	−	+	+
II_{19}	3	F	—	33	60	—	12.6	7.0	S-thal	O	+	+	−	−	+	−	+	+
III_1	10	M	—	94.9	—	—	0.8	5.1	A-thal									
III_2	5	F	35	67	—	33	0.8	—	AC	A_2B	+	+	−	−	−§	−	+	+

* Hemoglobin F is included with hemoglobin A_1 in starch block separations.
† Alkali denaturation technique.
‡ Paper electrophoresis only.
§ D^u negative also.

1959) and by brilliant biochemical investigations which disclosed the precise biochemical abnormality of hemoglobins S and C by means of the molecular dissociation-reassociation procedure (Rhinesmith, Schroeder, and Pauling, 1957) and the "fingerprint" technique (Ingram, 1957; Hunt and Ingram, 1958). The hemoglobin molecule was found to consist of heme and two pairs of chemically distinguishable polypeptide chains. The globin portion may be schematically designated $\alpha_2\beta_2$. The sole difference between hemoglobins S and C was the replacement of the same glutamic acid (#6 of the β chain) of hemoglobin A by valine in hemoglobin S and lysine in hemoglobin C. The rest of the molecular structures were identical. In accord with the most recent amendment to the nomenclature of abnormal hemoglobins, these two proteins may now be designated $\alpha_2\beta_2^{6\ val}$ (hemoglobin S) and $\alpha_2\beta_2^{6\ lys}$ (hemoglobin C) (Gerald, 1961). The demonstration that the change was at the same position in the hemoglobin molecule strongly supported allelism of the genes responsible for synthesis of hemoglobins S and C.

The major phenotypic expression of a thalassemia gene is impairment of synthesis of hemoglobin A_1 resulting in hypochromic anemia. Since the hemoglobin

molecule consists of three chemically different subunits—namely, heme groups and α and β polypeptide chains, a "thalassemia" might result from impaired fabrication of any of these subunits.

Abnormalities of heme synthesis have been described in experiments with thalassemic erythropoietic cells in vitro (Bannerman, Grinstein, and Moore, 1959), but the significance of these observations is not clear.

A fairly well defined thalassemia variant has been postulated to result from faulty synthesis of α chains. In the heterozygous state of this condition mild hypochromic microcytic anemia is seen. Hemoglobin A_2 is present in normal or decreased concentrations. When the gene for this form of "α thalassemia" interacts with the genes for hemoglobin S or C, which have abnormal β chains (Itano, Singer, and Robinson, 1959), there is no increase in the proportion of the abnormal hemoglobin above that observed in patients with the heterozygous hemoglobin trait. A few pedigrees suggest that the "α thalassemia" gene is not allelic to those responsible for synthesis of hemoglobins S and C (Ingram and Stretton, 1959).

Evidence to date suggests that the most common form of thalassemia—at least in Caucasians—is associated with a quantitative defect in synthesis of the β polypeptide chain. This form of "β thalassemia" may be characterized electrophoretically by elevation of the percentage of hemoglobin A_2. This protein contains normal α chains and a new chain designated δ ($\alpha_2\delta_2$), which has an amino acid sequence nearer to that of the β chain than the α (Muller and Jonxis, 1960). A secondary property is interaction with the genes responsible for abnormal β chain hemoglobins so that the red cells of the double heterozygote contain more than 50 per cent of the abnormal hemoglobin (Ingram and Stretton, 1959).

The S hemoglobin observed in the pedigree reported above had the sickling and agar electrophoretic properties of ordinary hemoglobin S, and the hemoglobin C in this family had a "fingerprint" pattern identical with that of the usual hemoglobin C ($\alpha_2\beta_2^{6\ lys}$) (We are grateful to Dr. Park S. Gerald, Boston, Massachusetts, for the "fingerprint" data on this hemoglobin), establishing that they are the common chain variants. The thalassemia observed in this family is associated with elevated hemoglobin A_2 and hypochromic microcytosis, thus fulfilling the criteria of "classical thalassemia" (Gerald and Diamond, 1958).

The mating I-3 × I-4 is the most informative as it contains a parent doubly heterozygous for thalassemia and hemoglobin C. If the genes governing synthesis of the β chains of hemoglobins S and C were allelic with or closely linked to the gene for "classical thalassemia", then four phenotypes would be possible in their children. These are CS, CA, A-thalassemia and S-thalassemia. If the "classical thalassemia" gene were widely separated from the other two, additional phenotypes would be possible. These would be C-S-thalassemia, C-thalassemia, AS, and A. Since none of the latter and all of the former occur in this pedigree, it is likely that the three genes are allelic or closely linked. The mating of II-1 × II-2 is also informative since neither child inherits both C and thalassemia from the doubly heterozygous mother. A close genetic relationship might be expected to exist between mechanisms of synthesis of either qualitatively

abnormal β chains (hemoglobins S and C) or quantitatively deficient β chains ("classical thalassemia").

SUMMARY

This report describes a Negro kindred in which the genes for hemoglobin C, hemoglobin S and "classical thalassemia" occurred singly and in various combinations. Children born from the union of a mother with hemoglobin C-thalassemia disease and a father with hemoglobin S trait were observed to have "classical thalassemia" trait, hemoglobin C trait, S-thalassemia or C-S disease. The union of a father with "classical thalassemia" trait and a mother with hemoglobin C trait, resulted in children with "classical thalassemia" trait, hemoglobin C trait, hemoglobin C-thalassemia and normal hemoglobin only. Two children of a woman with C-thalassemia married to a normal man had hemoglobin C trait and "classical thalassemia".

The observations in this pedigree add support to the hypothesis that the genes associated with hemoglobin S, hemoglobin C and "classical thalassemia" are either very closely linked or true alleles.

REFERENCES

Bannerman, R. M., Grinstein, M., and Moore, C. V. 1959. Haemoglobin synthesis in thalassaemia; *in vitro* studies. *Brit. J. Haemat.* 5: 102–120.

Gerald, P. S. and Diamond, L. K. 1958. The diagnosis of thalassemia trait by starch block electrophoresis of the hemoglobin. *Blood* 13: 61–69.

Gerald, P. S. 1961. Lecture at A Short Course in Human Genetics, Bar Harbor, Maine. August 7–18.

Hunt, J. A. and Ingram, V. M. 1958. Allelomorphism and the chemical differences of human haemoglobins A, S and C. *Nature* 181: 1062–1063.

Ingram, V. M. 1957. Gene mutations in human haemoglobin: the chemical difference between normal and sickle cell haemoglobin. *Nature* 180: 326–328.

Ingram, V. M. 1961. Hemoglobin and its abnormalities. Springfield: Charles C Thomas. p. 153.

Ingram, V. M. and Stretton, A. O. W. 1959. Genetic basis of the thalassaemia diseases. *Nature* 184: 1903–1909.

Itano, H. A. 1953. Qualitative and quantitative control of adult hemoglobin synthesis. A multiple allele hypothesis. *Am. J. Human Genet.* 5: 34–45.

Itano, H. A., Singer, S. J., and Robinson, E. 1959. Chemical and genetical units of the haemoglobin molecule. In *Biochemistry of Human Genetics.* CIBA Foundation Symposium. Boston: Little, Brown and Co. pp. 96–111.

Muller, C. J., and Jonxis, J. H. 1960. Identity of haemoglobins A_2. *Nature* 188: 949–950.

Neel, J. V. 1958. Genetic aspects of abnormal hemoglobins. In *Conference on Hemoglobins,* Publication #557. National Academy of Sciences—National Research Council. Washington, D.C., pp. 253–271.

Nomenclature of abnormal hemoglobins. 1960. *J. Am. Med. Assoc.* 174: 1845–1846.

Pearson, H. A. and McFarland, W. 1959. Starch block electrophoresis of hemoglobins. *U. S. Armed Forces Med. J.* 10: 693–700.

Ranney, H. M. 1954. Observations on the inheritance of sickle cell hemoglobin and hemoglobin C. *J. Clin. Invest.* 33: 1634–1641.

Rhinesmith, H. S., Schroeder, W. A., and Pauling, L. 1957. A quantitative study of the hydrolysis of human dinitrophenyl (DNP) globin: the number and kind of polypeptide chains in normal adult human hemoglobin. *J. Am. Chem. Soc.* 79: 4682–4686.

Singer, K., Chernoff, A. I., and Singer, L. 1951. Studies on abnormal hemoglobins, I. Their

demonstration in sickle cell anemia and other hematological disorders by means of alkali denaturation. *Blood* 6: 413–428.

SMITH, E. AND CONLEY, C. L. 1956. Quoted in Zuelzer *et al.*, 1956.

SMITH, E. W. AND KREVANS, J. R. 1959. Clinical manifestations of hemoglobin C disorders. *Bull. Johns Hopkins Hosp.* 104: 17–43.

ZUELZER, W. W., NEEL, J. V., AND ROBINSON, A. R. 1956. Abnormal hemoglobins. In L. M. Tocantins [ed.] *Progress in Hematology*, Volume I. New York: Grune and Stratton. pp. 91–137.

Relations Between the ABO, Secretor/ Non-Secretor, and Lewis Systems with Particular Reference to the Lewis System

P. H. ANDRESEN*

Bispebjerg Hospital, Copenhagen, Denmark

THE PHENOTYPIC and genotypic interpretations of the blood groups have gradually proved extremely complicated. If the *description could be simplified* or if the different findings could be *classified better*, much would be gained.

It must always be borne in mind that despite this complexity, the blood groups are among the simplest biological phenomenon in man, that despite the numerous phenotypic variations they can be collected within fixed limits. It has been clearly demonstrated by more than 100,000 investigations that they are transmitted by simple heredity, although of course a large number of *curiosities* have been encountered within such a vast collection of material. Most of these curiosities serve merely to remind us of the multiplicity of nature, but *exceptional variants* give clues to the unravelling of the maze of heredity.

The following presentation is not a complete description of the systems, and therefore the material is somewhat inhomogeneous—other facts might have been used in the description.

The ABO, Lewis, and secretor/non-secretor systems are now considered an entity in which all antigens are chemically related, but in which independent systems of genes determine the phenotype.

While the heredity of the ABO system and the secretor/non-secretor system must be considered as established, the heredity and the entire structure of the Lewis system is still a matter of discussion.

The phenotypes within these systems comprise reactions with:

(1) antigens attached to the red cells or to other cells;

(2) antigens dissolved in plasma, saliva, and other secretions.

The main antigens are:

(a) The antigens of the ABO system; A (variants to be mentioned later), B, and H. H is an antigen closely related to the others, whose occurrence is, however, unrelated to the genes of the ABO system.

(b) The antigens of the Lewis system, Le^a, Le^b, Le^{b_2}, Magard factor, and Le^x. These antigens have been studied particularly in the red cells and in their water soluble form in saliva and other secretions.

Received June 19, 1961

* Aided by grants from the Danish Foundation for the Advancement of Medical Science and from the Ciba Foundation.

The presence or absence of the water soluble antigens A, B, or H in saliva and certain other secretions divide humanity into two groups: secretors and non-secretors.

Less work has been done concerning the presence of the antigens in cells other than the red blood cells, but A, B, and H antigens are present in an alcohol soluble form in most cells (24). During the past few years, our knowledge regarding the antigen content in the cell has been extended by the use of antibodies with fluorescent stains, Glynn and Holborrow (18).

Still less is known about the content of antigens in the plasma and serum, although the presence of the antigens of the Lewis system in these body fluids is of decisive importance.

Before proceeding, it would be well to recapitulate some of the most important facts concerning the Lewis system.

THE LEWIS SYSTEM

A. PHENOTYPE

Red cells:

In 1946 Mourant (34) found an agglutinin (anti-Le^a) in the serum from a woman, Mrs. Lewis. This serum agglutinated about 24 per cent of the persons studied [Le(a+)], regardless of their ABO group.

In 1948 Andresen (4) demonstrated that this blood group property was presumably inherited as a recessive character and that its distribution differed in babies and adults.

In 1947 Andresen (5) found an agglutinin, anti-Le^b, reacting with most blood cells, which did not react with the Le^a agglutinin [Le(a−b+)], about 10 per cent of the samples failed to react with either of these sera [Le(a−b−)]. However, anti-Le^b gave a weaker reaction with A_1 cells, so that the percentage of Le(a−b−) was higher within group A_1.

In 1948 Grubb (19) showed that saliva from most people (about 90 %) contains a substance (Le^a substance) characterized by binding anti-Le^a and thus preventing the agglutinin from reacting with blood cells of group Le(a+).

At the same time, Grubb found that all persons of blood group Le(a+) were non-secretors of ABH substance, whereas their saliva contained large quantities of Le^a substance.

Lastly, Grubb demonstrated that saliva from all persons, including those of group Le(a−b−), who were secretors of ABH substance, also contained a substance, *Le^b*, which prevented anti-Le^b from agglutinating Le(a−b+) cells.

In 1949 Andresen and Jordal (7) discovered an agglutinin (anti-Le^x), which agglutinated all blood cells except those, which failed to react with both anti-Le^a and anti-Le^b. This gives blood group Le(a−b−)X−. Anti-Le^x nearly always co-exists with anti-Le^a, and only a few sera containing anti-Le^a are completely devoid of anti-Le^x. This is the reason why it is so often impossible to obtain completely negative reactions with anti-Le^a, except with blood cells of group Le(a−b−)X−. Many workers assume that anti-Le^x is a mixture of anti-Le^a and anti-Le^b.

In 1951 Grubb (20) elaborated his studies, showing that in all persons whose saliva did not contain Le^a substance, the blood cells were group Le(a−b−) and that this group must be identical with that, which Andresen, Andersen, Jordal, and Henningsen (8) had called Le(a−b−)X−.

In 1950 Brendemoen (13) discovered another agglutinin reacting with blood cells of group Le(a−b+), but which is not identical with anti-Le^b (Andresen) and which was later designated anti-Le^{b_2}. The assumption is that it reacts with another receptor and is not inhibited by Le^b substance in the saliva from persons of group Le(a−b−), who are secretors. On the other hand it is inhibited by saliva from some group Le(a+b−) individuals. Another peculiarity is that this receptor is not weaker in group A_1 persons.

Since these two antibodies have not been clearly distinguished, some confusion has resulted.

In 1952 Jordal and Lyndrup (27a) demonstrated that all newborn infants (cord bloods) are group Le(a−b−), but that 90 per cent show group Le(X+), i.e. the same distribution of Le(X+) as in adults.

In 1955 it was shown by Sneath and Sneath (40) that blood cells of group Le(a−b+) suspended in plasma from group Le(a+b−) persons will absorb Le^a substance, so that they test Le(a+b+).

In 1956 Mäkelä and Mäkelä (35) in a similar way demonstrated that the Le^b substance from plasma may be absorbed and will convert Le(b−) cells to Le(b+). However, this conversion did not take place, if Le(b−) cells were suspended in a fluid containing Le^b substance from saliva.

In 1958 J. Andersen (2) discovered an agglutinin, which reacted with a receptor (the Magard receptor) present only in the red cells of group A, Le(a−b−) persons, who are secretors.

Since this antibody appears to be combined with a very weak α_1, it is difficult to decide from the experiments whether the receptor is present also in saliva.

If the receptor is present in saliva, it is present in all group A secretors.

In 1960 it was shown by Levine and Celano (28) that it is also not possible to convert Le(a−) cells to Le(a+) by Le^a substance from saliva. On the other hand, this conversion could be accomplished by using tanned red cells of group Le(a−) placed in fluid containing Le^a from saliva. By means of these converted red cells Levine and Celano could immunize rabbits, which then formed anti-Le^a antibodies.

B. HEREDITY

On the basis of family studies of red cells tested with anti-Le^a and anti-Le^b, the theory advanced by Andresen (5), that Le(a+) is transmitted as a recessive character, was the first to be generally accepted.

According to this theory each person's Lewis group depends upon the two allelic genes *Le^a* and *Le^b*, Mourant's original Lewis group Le(a+b−) having genotype *Le^a Le^a*, and group Le(a−b+) genotype *Le^a Le^b* or *Le^b Le^b*. The occurrence of phenotype Le(a−b−), however, could not be explained by this theory. Andresen *et al.* (8) therefore, extended the genetic theory—after having

demonstrated the character Le(X+), by introducing a gene Le^x which was dominant and governed not only Le(X+), but also the occurrence of receptors Le^a and Le^b in red cells. Consequently group Le(a−b−)X− must have genotype $Le^z\ Le^z$. The pair of alleles Le^x and Le^z was independent of the Lewis genes as well as of the genes of the ABO system.

All family series published so far are formally in agreement with the theory, especially after it was shown by Ceppellini (15) and J. Andersen (3) that matings of the combination Le(a+b−) × Le(a+b−) may give rise to children of group Le(a−b−), who are non-secretors.

Grubb's studies on the occurrence of Le^a and Le^b substance in saliva, however, militated against the theory, as it could not explain the presence of Le^b substance in Le(a−b−) secretions. In particular, Grubb (20) found that genes Le^a and Le^b could not possibly be allelic and therefore put forward a theory based on a dominant allele, Le^a, which determined the presence of Le^a substance in the secretions, and an allelic gene l indicating the alternate of the Le^a allele.

To be able to explain the occurrence of Mourant's Lewis groups and the relation between the secretor/non-secretor characters, Grubb (like Andresen and later Cepellini) had to operate with two genetically independent gene systems, one of which corresponded to the secretor/non-secretor genes.

Many workers have tried to explain the heredity of the Lewis system on the assumption of three alleles. As already stressed by Andresen *et al.* (8) 1950, this is not possible, if also the relation to the secretor/non-secretor system is to be explained.

Wiener (46) tried to set up a theory operating with three alleles, considering only the phenotypes of the red cells. This theory corresponded to the percentage distribution of the groups and to the results of the family series available at that time. Wiener's theory was overthrown by Ceppellini's and Andersen's finding that mating of two Le(a+) persons might produce children of group Le(a−), as expected by Grubb and by Andresen. Sneath and Sheath's demonstration that mating Le(a−b−) × Le(a+b−) persons might give rise to Le(a−b+) children would also be at variance with Wiener's theory.

Since Ceppellini could not accept all of Grubb's results in regard to the presence of receptor Le^b in saliva, he tried to modify Grubb's theory. Ceppellini (15) stressed the fact that saliva from group Le(a−b−) secretors did not inhibit anti-Le^b of the type Brendemoen (= Sneath's anti-Le^{b_2}), and thought therefore, that Grubb's anti-Le^b reacted partially as an anti-H. This opinion has been clearly contradicted by Grubb. Incidentally, it has been unambiguously demonstrated by Ceppellini as well as by Jordal, and by Sneath and Sneath that saliva from Le(a−b−) secretors contains a substance, which inhibits anti-Le^b of the type demonstrated by Andresen.

In point of fact, Ceppellini's, Grubb's and Andresen's theories are identical, the only difference relating to the explanation of the Le^b receptors and substances.

This difference, however, is of decisive significance in relation to the biochemical interaction in the formation of the various receptor substances. This question will, therefore, be considered in more detail later.

Grubb (20) assumed, even at an earlier date, that the presence of the blood cell characters Le(a+b−) and Le(a−b+) were due to the blood cells taking up Le^a and Le^b substance from the plasma, group Le(a−b−) being due to absence (or deficiency) of the substances in the plasma. He considered that the Le(a−b−)X− group described by Andresen *et al.* was identical with the group lacking Le^a substance in the saliva. If so, Grubb's Le^a gene and Andresen *et al.*'s Le^x must be the same and the two genetic theories identical. In order to unite the two theories it is necessary to establish that Andresen and Jordal's anti-Le^x is in fact a specific agglutinin and not a mixture of anti-Le^a and anti-Le^b.

(1) *A priori*, it is unlikely that anti-Le^a and anti-Le^b will occur in the same person.
   (a) Anti-Le^a has been found only in persons, who are Le(a−b−) and secretors of ABH substance, Jordal (27), Miller *et al.* (30). Typical anti-Le^x always co-exists with anti-Le^a, most anti-Le^a containing a major or minor quantity of anti-Le^x.
   (b) Anti-Le^b (Andresen) has been demonstrated only in non-secretors of group Le(a−b−). Brendemoen (13) demonstrated anti-Le^b in non-secretors of group Le(a+b−), but this was anti-Le^{b_2}.

(2) Jordal's (26, 27a) investigations revealed that blood cells of newborn infants are divisible, by means of anti-Le^x, into the two groups Le(a−b−)X+ and Le(a−b−)X−, corresponding exactly to this classification in adults. In others words, this classification can be made despite the fact that the blood cells of newborn infants fail to react with either anti-Le^a, anti-Le^b, or anti-Le^{b_2}.

Consequently, *anti-Le^x must be considered as a specific agglutinin*, which has a corresponding specific receptor X closely related to the Le^a substance. The presence of receptor X in the blood cells is always accompanied by the presence of Le^a substance in the secretions. The presence of the X receptor is entirely independent of the subject's secretor/non-secretor status. There is no correlation between the X receptor and Le^b substance.

These were the most important experimental results obtained by using typical anti-Le^a, anti-Le^x, anti-Le^b, and anti-Le^{b_2} sera.

If an attempt is made to classify the various phenotypic findings into not one, but *two independent systems*, a number of the difficulties are overcome. The reason why such a clear division has been avoided so far is that, although independent, these two systems are based largely upon the presence or absence of the same chemical substance: the Le^a substance.

In the following, it is of decisive importance to interpret Le^x [Andresen and Jordal (7)] as a specific receptor and to consider this receptor and the occurrence of Le^a in secretions as a manifestation of the effect of the same gene *Le* (= *Le^a*: Grubb = *Le^x*: Andresen and Jordal).

The first system, which might be called the Lewis substance system, has two phenotypes. One is characterized by the presence of Le^a substance in the secretions and by the red cells reacting with anti-Le^x. The other lacks Le^a substance in the secretions, and the red cells are not agglutinated by anti-Le^x. (From the

absence of Le^a substance it also follows that the red cells must be of phenotype Le(a−b−), *vide infra*.)

The second system comprises, as far as the red cells are concerned, the original Lewis system with groups Le(a+b−) [= Mourant's Lewis group], Le(a−b+), and Le(a−b−). The Le(b+) group may be demonstrated with anti-Le^b (Andresen) as well as anti-Le^{b_2} (Brendemoen). This, the original Lewis system, is thus, as shown by Grubb (20), a specific phenotypic manifestation on the part of the blood cells, of the already known secretor/non-secretor system, the blood cells taking up from the plasma some of the substances, which are formed in the glands or other cells.

All the phenotypic factors relating to the secretor/non-secretor system are explicable on the basis of the conversions caused by the dominant *Se* gene in the glands.

Se gives rise to conversion of the ABH blood group substances present in the glands and many other cells to a water soluble form. In the course of this process new water soluble substances are formed, especially Le^b, Le^{b_2}, and Magard substance.

These activities are more complicated than was perhaps assumed initially. It is worth emphasizing that originally the secretor/non-secretor system was characterized exclusively by the secretion or non-secretion of water soluble ABH substances. Studies of numerous families confirm heredity determined by two alleles, the dominant *Se* and the recessive *se*. Since the original Lewis system is a manifestation of this inherited system, its heredity is explained by the same genes, and *Se* is therefore = Le^b (Andresen) and *se* = Le^a (Andresen).

The only difficulty in understanding the heredity and phenotypes of the original Lewis system is now easily solved, the blood group character Le(a+) in non-secretors being present only in persons, who are able to form Lewis substances at all (i.e., those who have the *Le* gene = Le^a (Grubb) = *X* (Andresen and Jordal). Blood group Le(a−b−) must then comprise secretors as well as non-secretors in the same ratio as in the general population (table 1).

Before discussing the formation of the various blood group substances, let us

TABLE 1. THE GENOTYPE AND PHENOTYPE OF THE SALIVA AND THE RED CELLS IN THE LEWIS SUBSTANCE SYSTEM AND IN THE ORIGINAL LEWIS SYSTEM

Lewis substance system				
Genotype	Phenotype			
Le^x-red cells Le^a-substance in saliva		Genotype	Orig. Lewis system phenotype	ABH secretor + non-secretor−
Le Le *Le le*	+	*Se Se* *Se se*	Le(a−b+)	+
		se se	Le(a+b−)	−
le le	−	*Se Se* *Se se*	Le(a−b−)	+
		se se	Le(a−b−)	−

have a further look at the H-antigen. By way of introduction, it may be emphasized that the H antigen present in the red cells is completely independent of the subject's secretor/non-secretor status and is an alcohol soluble form of H. In addition to this alcohol soluble form of the H antigen, there is, as already mentioned, a water soluble form in saliva and other secretions. The occurrence of the antigen depends upon gene *Se* (38). Whether the different H receptors are identical is not known. As early as 1948 Morgan and Watkins (see 44) pointed out that agglutinins reacting with group O blood cells could be divided into two categories. One was called anti-H, since the agglutination was completely inhibited by the water soluble H antigen; the other was called anti-O. As demonstrated by Sanger (37), anti-H occurs only in serum from persons of group Le(a+b−), i.e. non-secretors, while anti-O may occur in secretors as well as non-secretors. This finding might possibly be interpreted to the effect that blood cells of group O have two receptors, whereas the water soluble antigen has only one. In the following, the H antigen of the blood cells will be designated $H^{1\text{-}2}$ and the water soluble one H^2.

A particularly strong anti-H occurs in persons of the so-called Bombay-group "O" whose blood cells are characterized by the absence of the A, B, and H substances (they also lack the Le^b group). With one exception reported by Simmons (39) all are Le(a+). Anti-H is as strong as iso-anti-A and B and also reacts at 37°. Ceppellini called attention to the very close relationship between the occurrence of water soluble H antigen and the occurrence of Le^b (Andresen) substance.

The heredity of the Bombay group was first explained theoretically by Ceppellini (14) on the assumption of an inhibitory gene. This theory has subsequently been elaborated by Levine's family studies. In one family there was an AB child, although its mother apparently was group O. Levine demonstrated that the mother was "O", and the father was group A. Levine explained that the mother must be genotype *xx BO*. The mother had the phenotype "O", because the phenotype B and O (reaction with anti-H) only will be developed with genotype *XX* or *Xx*. [Watkins has later proposed *H* and *h* (42)].

Closely related to the H antigen are the numerous variations of blood group A. I shall not go into these peculiarities which—though some must be designated as curiosities—may acquire great significance, e.g. in medico-legal decisions [van Loghem (29)]. Only the variant first designated A_x by Gammelgaard (17) and later A_m by W. Weiner (45), will be mentioned. What characterizes this group is an ample content of A substance in the saliva, while the A character is practically undemonstrable in the blood cells. Weiner's family studies explained the heredity by assuming the existence of a dominant gene, *Y*, necessary for the transfer of the A character to the blood cells. The genotypes of A_m must be *yy* and in this case the A substance cannot be transferred to the blood cells. A similar variant of group B was found by Armstrong, Gray, Race, Sanger, and Thompson (10): The blood group was determined as O, while the saliva contained ample B substance and the serum no anti-B.

Levine's investigations into the Bombay group and Weiner's explanation of

the A_m group have extended the picture representing the heredity of the ABO system. Race and Sanger (36) have brought this view up to date (1958), and later Watkins and Morgan (44) have dealt with the question from a biochemical point of view in their paper "Possible Genetical Pathways for the Biosynthesis of Blood Group Mucopolysaccharides" (1959). Thereby, these authors have opened up a possibility for biochemical-serological consideration of the phenotypic factors with a view to the heredity.

The groups of atoms which have the specific immune properties constitute only a small proportion of the macromolecules which make up the blood group substance. Various receptors may be attached to one of these molecules or aggregates of these molecules. Many findings indicate that the same secretion may contain uniform macromolecules with different combinations of the specific groups (31, 42). Watkins and Morgan (44) were the first to try to apply this knowledge in a schematic illustration of the interaction between these chemical substances and the blood group genes. The blood group substance is assumed to be formed when enzymes, corresponding to the blood group genes, act upon a human non-antigenic ground substance (precursor substance) or compounds derived from it.

I shall now try to describe Watkins and Morgan's theory and Ceppellini's (16) extension of this theory to comprise also the group characters of the red cells (similar to Watkins). However, I have modified the schematic presentation to be able to apply it also to the views propounded in this paper.

The precursor substance is assumed to be made of a number of uniform macromolecules in a fairly loose chain, here designated as: — — —, while the derived specific compounds are designated: A A A or Le^a Le^a Le^a or A A H or AH AH, etc.

Since the biochemical properties have been elucidated in any detail only in the case of the water soluble blood group antigens, Watkins and Morgan's scheme comprises only these antigens. They use Ceppellini's (15) theory concerning the Lewis system. Thus, in addition to the *A*, *B* and *O* genes of the ABO system, they include the *L*, *l*, and genes as interpreted by Ceppellini. In consequence, Le^b in Watkins and Morgan's scheme indicates a receptor binding anti-Le^b (Brendemoen) and thus not identical with the receptor, which binds anti-Le^b (Andresen). Therefore, Watkins and Morgan's Le^b will be designated here as Le^{b_2}.

Table 2 illustrates the processes, which Watkins and Morgan believe are required to explain the formation of the water soluble antigens from the precursor substance. Watkins and Morgan, however, put forward two suggestions to solve the problem. The former comprises only processes 1–6 according to which H could be formed only by Le^a Le^a Le^a + S' = H(+Le^{b_2}). In order to satisfy the demands made by the known genetic series, it is necessary, however, to assume also the possibility of the process — — — +S = H H H. Gene S' must then be able to transform both Le^a and precursor substance to H receptor.

The substances, which one might expect to find in the secretions according to

TABLE 2. WATKINS AND MORGAN: "POSSIBLE GENETICAL PATHWAYS—" (44). THE FIRST PART OF FIG. 1 (p. 109) AND 2 (p. 110) IN A SLIGHTLY MODIFIED FORM. NOS. 1–6 REPRESENT FIG. 1, NOS, 1, 2, 3, 5, 6 AND 7 FIG. 2. THE TABLE SHOWS THE DIFFERENT INTERACTIONS BETWEEN SUBSTANCE AND GENES

No.	Substance	+	Gene	=	Converted to substance	+	Unconverted substance
1	— — —		*L′*		Le^a Le^a Le^a		
2	Le^a Le^a Le^a		*S′*		H H Le^{b_3} Le^{b_2}		Le^a
3	— — —		*l′*				— — —
4	— — —		*S′* or *s′*				— — —
5	Le^a Le^a Le^a		*s′*				Le^a Le^a Le^a
6	H H H		*A* (*B O*)		A A A		H H
7	— — —		*S′*		H H H		

Precursor substance: — — —
Le^a substance: Le^a Le^a Le^a
ABO genes: *A B O*
Lewis system genes: *Le′* and *l′* (Ceppellini)
Secretor/non-secretor genes: *S′* and *s′*

H substance: H H H
A substance: A A A

TABLE 3. WATKINS AND MORGAN: "POSSIBLE GENETICAL PATHWAYS —" (44). SECOND PART OF FIG. 1 AND FIG. 2 IN A SLIGHTLY MODIFIED FORM, INCLUDING THE SUBSTANCES WHICH ACCORDING TO TABLE 1 WILL BE FOUND IN THE SECRETIONS FROM THE VARIOUS GENOTYPES. A COLUMN SHOWING THE PHENOTYPES OF THE BLOOD CELLS IS ADDED (ONLY A, THE SAME FOR B AND O). 2 CORRESPONDS TO WATKINS AND MORGAN'S THEORY 1, 2A TO THEORY 2

No.	Genotype AA or AO	Secretor + non-secretor −	Substances in the secretions	Blood group (P.H.A.)
1	*L′L′ S′S′* or *S′s′* or *L′l′ S′S′* or *S′s′*	+	A H Le^a Le^{b_2}	A Le(a−b+)
2	*l′l′ S′S′* or *S′s′*	−	"inactive substance Fl."? — — — —	A Le(a−b−)
2a	*l′l′ S′S′* or *S′s′*	+	A H Le^a Le^b	A Le(a−b−)
3	*L′L′ s′s′* or *L′l′ s′s′*	−	Le^a Le^a Le^a	A Le(a+b−)
4	*l′l′ s′s′*	−	"inactive substance Fl."? — — —	A Le(a−b−)

this theory are listed in table 3, the occurrence being given separately for each of the possible genotypes. Table 3 corresponds exactly to Watkins and Morgan's scheme, except that it includes also the blood groups, which will be found in each case. — — — has not been definitely demonstrated in saliva, but in an ovarian cyst, Watkins and Morgan found a substance, "inactive substance Fl", which must be assumed to be identical or closely related to the precursor substance.

TABLE 4. CEPPELLINI'S "SCHEME OF THE METABOLIC PATTERNS WHICH LEAD TO THE SYNTHESIS OF ABH ANTIGENS OF THE RED CELLS" (16) IN A SLIGHTLY MODIFIED FORM

Substance	+	Gene	Converted to Substance	+	Gene	Converted to Substance
— — —		*X* Levine	H H H		*B*	B B B group B
— — —		*X*	H H H		*O*	H H H group O
— — —		*x x*	— — —		*B*	— — — group "O"

Watkins has tried to apply these theories (42) also to the group properties of the red cells, but emphasizes: While it seems fairly certain that the actual group specific structures on the red cell antigens will be chemically identical with those on the water-soluble substances, it is not known whether these specific structures are part of the same type of mucopolysaccharide molecules as are found in secretions. Here Watkins stresses the significance of H substance in the red cells and the importance of the *X* gene (Levine) for which the designation *H* gene has been proposed.

Although the biochemical features are not known in detail, Ceppellini (16) too has tried to extend Watkins and Morgan's considerations also the group characters of the red cells on the basis of serological and genetic views. Since Ceppellini also included the Bombay group, he introduced the *X* gene (Levine). In fact, Ceppellini's elucidation in respect to the secretions corresponds exactly to Watkins and Morgan's, and there is no reason to go into it in more detail, although here too he has introduced the *X* gene as a complementary gene in connection with *S* in order to account for "O".

Table 4 gives Ceppellini's scheme of the red cell types. What is particularly notable is that as far as the blood cells are concerned he believes that *X* acts upon — — —, converting it into H H H. According to this theory, therefore, H may be formed in three ways: *X* is able to transform precursor substance, and *S'* will be able to transform both Le^a and precursor substance to H receptor. Since in Ceppellini's opinion all the Lewis groups in the blood cells have been formed in glands and other cells and have been transmitted through plasma to the red cells, the *Le* gene is not included in his account of the red cells.

In order to set up the theory advanced in the present paper in a corresponding schematic form, I have included all known blood group expressions and the presence of the alcohol soluble ABH receptors in the glands and other cells. Like Ceppellini's, the present theory is based exclusively upon serological and genetic results, but it is supported by the possibilities opened by Watkins and Morgan's biochemical results. The simplest assumption seems to be that all the group specific structures are derived from the same precursor substance and that the difference between the water soluble and alcohol soluble blood group substances is due to a binding of the specific structures to different molecules. Thus, the precursor substance must be assumed to be a macromolecule, large it is true, but not as large as the molecules of the water soluble blood group substances.

Of the precursor substance we know nothing apart from the fact that under the influence of the blood group genes it may be transformed to serologically active specific structures demonstrable by specific agglutinins and other antibodies. The "inactive substance F1" mentioned by Morgan (32) is presumably a binding of precursor substance and the mucopolysaccharides formed by the glandular cells.

The following five, genetically independent groups of genes are included in the ABO, Lewis substance, secretor/non-secretor system: *X x* (Levine), *A B O* (Bernstein), *Y y* (Weiner), *Se se* (Shiff), and *Le le* (Grubb, Andresen).

The series of processes required before all group substances have been formed must be divided into three groups: (1) initial conversions which must be assumed to proceed in the same way in haematopoietic tissues and other cells, especially in glands; (2) processes which take place only in the haematopoietic tissues, and lastly; (3) processes which take place in the glands and other cells in order to form the water soluble, secretable group antigens.

The point on which this theory differs from the others is the completely independent placement of the antigens of the Lewis system, arising through the action of the *Le* gene upon the precursor substance. All the other group substances are derived in some way or other from the conversion by the *X* gene of the precursor substances to H antigen.

Table 5 illustrates the course of the initial processes in all cells which can form group specific antigens.

(1) Gene $X = H$ (Morgan) influences the precursor substance, resulting in the formation of $H^{1\text{-}2}H^{1\text{-}2}H^{1\text{-}2}$ substance.
(2) Lacking *X* (genotype *xx*), no $H^{1\text{-}2}$ will be formed.
(3) Gene *Le* acts upon the precursor substance, forming Le^x substance.
(4) Lacking *Le* [genotype(*lele*)], no Le substance will be formed.
(5) Next, due to the action of genes *A* or *B* of the ABO system a major or

TABLE 5. THE VARIOUS INTERACTIONS BETWEEN PRECURSOR SUBSTANCE AND ITS DERIVATIVES AND THE DIFFERENT GENES, INCLUDING THE PROCESSES WHICH TAKE PLACE BOTH IN THE HAEMATOPOIETIC TISSUE, THE GLANDS, AND CERTAIN OTHER CELLS. THE PROCESSES RESULT IN THE PRESENCE OF SUPPOSED ETHANOL SOLUBLE ANTIGENS IN THESE CELLS

No.	Substance	+	Gene	=	Converted to substance	Rest of unconverted substance
1	— — —		*X* (Levine)		$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$	
2	— — —		*x x*			— — —
3	— — —		*Le*		$\underline{Le^x}\ \underline{Le^x}\ \underline{Le^x}$	
4	— — —		*le le*			— — —
5	$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$		*A* (*B*)		$\underline{A}\ \underline{A}\ \underline{A}$	$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$
6	$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$		*O*			$\underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}\ \underline{H^{1\text{-}2}}$

TABLE 6. THE PROCESS DEMONSTRATED BY WEINER IN WHICH GENE Y TRANSFERS THE A ANTIGEN TO THE RED CELLS. IT MUST BE ASSUMED THAT GENES CORRESPONDING TO THE B, H, AND Le^X ANTIGENS EXIST

Substance found in the haematopoietic cells	+	Genotype	Antigen acquired by the red cells
A A A		*YY* or *Yy*	blood group A
A A A		*yy*	no reaction with anti-A

TABLE 7. HOW—INDEPENDENTLY OF THE BLOOD GROUP GENES—Le^X IS CONVERTED TO WATER SOLUBLE Le^a SUBSTANCE IN THE GLANDS AND SECRETED

Substance	By the secretion of the glands converted to
Le^X Le^X Le^X	Le^a substance = Le^X − M

M = muchopolysaccharide macromolecules from the cells.

minor portion of H^{1-2} will be converted into A or B, while *OO* will preserve all H^{1-2} substance formed. Of the named blood group substances at least A and B are alcohol soluble.

The subsequent processes differ in the different cells.

Table 6 is meant to illustrate the process whereby the formed antigens are transferred to the red cells. This is known only for the A antigen in which the presence of the *Y* gene (Weiner) is necessary for the red cells to develop the A character. It is reasonable to assume that corresponding genes are required for the transmission of the other group properties. At any rate, as already mentioned, a person has been found, whose saliva contained ample B substance, while his red cells did not react with B-agglutinin.

The formation of the water soluble blood group substance is more complicated and must be assumed to form a link in the formation of glandular secretion. All the above-mentioned secretions contain varying quantities of mucopolysaccharides (now designated as M), and it must be considered likely that the water soluble blood group substances are formed by a binding of the named H^2, A, B, Le^b, Magard and Le^x substances to M as a link in cellular function. There is an essential difference between the ability of the A, B, H^{1-2} receptors and of the Le^x substance to form these compounds. While Le^x can enter into the metabolism of the cells, Le-M = Le^a substance being formed, the other receptors cannot enter into such a metabolism until they have been converted by the action of the *Se* gene.

The formation of Le^a substance is illustrated in table 7. The assumption affords a natural explanation of why Le^a substance can always be found as soon as *Le* is present. Provided that ample Le^a substance is formed (partly in *se se* individuals and partly in babies in whom the secretor ability is not yet fully developed), it passes on to the blood and is bound to the blood cells (it must be considered doubtful whether this is simple physical binding).

The next sphere comprises the formation of the other water soluble antigens designated as: A, B, H^2, Le^b, Le^{b_2}, and the Magard factor. The formation of these antigens is more complicated than one might perhaps have expected. Gene *Se* reacts only with A B H^{1-2}, but the ultimate result depends upon the presence or

TABLE 8. THE INTERACTION BETWEEN GENE *Se* AND *se* AND THE FORMED BLOOD GROUP ANTIGENS IN PERSONS OF GROUP Le(a−b−) (GENOTYPE: *le le*). MACROMOLECULE OF MUCOPOLYSACCHARIDE M COMBINED WITH THE RECEPTORS, M−

Antigen substance	+	Gene	=	Antigen cells metabolism gives the water soluble antigen M−	Secreted in	
					Secretion M−	Plasma M−
A A A		*Se*		A A A + Magard receptor	A + Magard-receptor	Magard-receptor
B B B		*Se*		B B B + ?	B + ?	
$H^{1-2}H^{1-2}H^{1-2}$		*Se*		H^2 H^2 H^2 + Le^b	H^2 + Le^b	
A A A B B B		*se se*		non-secretor of A, B, H, Le^b and Le^a		
$H^{1-2}H^{1-2}H^{1-2}$				"inactive substance Fl"?		

absence of Le^x. It must be assumed that *Se* does not react directly with Le^x, since Le^b has not been demonstrated in group "O", which may occur in the presence of the *Se* gene. That a close relationship exists between Le^x and *Se* is apparent from the fact that the amount of Le^a substance, which forms is inversely proportional to the manifestation of *Se*. This phenomenon is particularly apparent in the gradual decrease of Le(a+) reactions in infants as they develop from 1 to 6 months of age.

Since the formation of A, B, and H^2 is independent of Le^x, the simplest procedure would be to consider first the conversions, which take place in the glands of Le(a-b-) persons, who are secretors. These processes are illustrated in table 8. It will be seen that in these cases water soluble antigens other than A, B, and H^2 are formed. This accords with the theories of Watkins and Morgan, and of Ceppellini. The present explanation of the Magard factor was indicated also by Ceppellini, and the formation of Le^b gives a natural explanation of the close relation between the occurrence of the Le^b receptor (Andresen) and the H^2 antigen as suggested several times by Ceppellini.

Tables 8 and 9 show in each individual case whether the water soluble antigens pass into the plasma. I feel that there may be reason to stress that no known relationship exists between the formation of water soluble antigens and their transfer to the plasma. Le^b does not pass into the blood, but the Magard factor does (table 8).

Before leaving genotype *le le*, I should like to emphasize that according to Race and Sanger the amount of water soluble ABH antigen in the saliva is particularly ample in this type of person, if he is a secretor.

Table 9 shows the interaction in cells, which contain both M and Le^a substances (genotype: *Le Le* or *Le le*). The ABH antigen converted by *Se* can be bound to M as well as to Le^a substance. By combining Le^a substance with Le^b and Magard receptors, the Le^a receptor will be converted to Le^{b_2} receptor, and the Magard

TABLE 9. THE INTERACTION IN THE GLANDS AND OTHER CELLS BETWEEN GENE *Se* AND *se se* IN PERSONS OF GENOTYPE *Le le* OR *Le Le*, GROUP Le(A−B+) AND Le(A+B−)

Antigen substance +	Gene	= Antigen cells metabolism gives the water soluble antigen M−	Secreted in	
			Secretion M−	Plasma M−
$\underline{H^{1\text{-}2}Le^X}$	*Se*	$H^2\ H^2\ \underline{Le^b\ Le^{b_2}}$	$H^2 + \underline{Le^b\ Le^{b_2}}$	$\underline{Le^b\ Le^{b_2}}$
$\underline{A\ Le^X}$	*Se*	$A\ \underline{Le^{b_2}}$	$A\ \underline{Le^{b_2}}$	$\underline{Le^{b_2}}$
$\underline{H^{1\text{-}2}Le^X}$	*se se*	the cells convert all $\underline{Le^X}$ to $Le^a = Le^X - M$	Le^a	Le^a
$\underline{A\ Le^X}$				

receptor will be lost: $Le^b + Le^a\ (Le^X - M) = Le^b - Le^{b_2} - M$, and Magard receptor + $Le^a\ (Le^X - M) = Le^{b_2} - M$.

Since a single macromolecule M can bind several receptor groups, many combinations may occur, e.g.

$$\begin{matrix} A \\ H^2 \end{matrix} \Big\} - M - Le^{b_2} \qquad H^2 - M - Le^{b_2} - Le^b \qquad H^2 - M \begin{matrix} - Le^{b_2} \\ - Le^a \end{matrix} - Le^b \text{ etc.}$$

A number of these combinations are known [Watkins (42)], and the explanation is entirely in keeping with Ceppellini's interpretation of the relation between H^2 and Le^b. The theory explains why the quantity of Le^a substance varies in inverse proportion to the manifestation of the *Se* gene, and why Le^b must vary with the amount of $H^{1\text{-}2}$.

SUMMARY

After introductory remarks on the ABHO, Lewis, and secretor/non-secretor system, the author discusses the most important serological findings which have been considered links in the Lewis system.

The description comprises the Le^a, Le^b, Le^{b_2}, X, and Magard receptors, especially the relation of these blood cell properties to the secretor/non-secretor system. It is emphasized that receptors Le^b and Le^{b_2} are serologically different receptors. Grubb's demonstration of the independent role of Le^a substances in the secretions is stressed.

The complicated theories on the inheritance of the Lewis system are reviewed. The importance of the H receptor is mentioned. It is stressed that in this respect also two properties have to be considered, viz. those which react with the so-called anti-O, and those which react with anti-H (H^1 and H^2).

After trying to prove the existence of receptor X (Andresen and Jordal), the heredity is further elucidated. The blood cell properties, which have been included in the Lewis system [the original Lewis system (Andresen)] are phenotypic properties governed by the secretor gene *Se*.

The presence of the Le^a substance in the organism is an independent, inherited

property determined by gene *Le* (Grubb). It is suggested that this system be designated *the Lewis substance system*. Since the Le^a substance plays an important role in the original Lewis system as well as in the Lewis substance system, the phenotypic conditions will always be conditioned by the combined effect of the genes of both systems.

After reviewing Watkins and Morgan's "Possible Genetical Pathways for the Biosynthesis of Blood Group Mucopolysaccharides" and Watkins' and Ceppellini's considerations regarding the application of certain theories to the group properties of the red cells, these ideas are applied to the theory advanced in the present paper.

It is assumed that all the group properties (receptors) of the named systems are derived from one precursor substance converted under the influence of the genes governing the various systems. Formation of the Le^x substances is an independent aspect governed by gene *Le*. On the other hand, the formation of H^{1-2}, A, and B substance (alcohol soluble as well as water soluble) and of Le^b, Le^{b_2}, as well as the Magard substance will pass through a more complicated process under the action of genes *X*, Levine (= H, Morgan, *A*, *B*, and Y (W. Weiner), and *Se*.

The formation proper of the water soluble blood group substances must be assumed to be the result of the cellular metabolism, since the serologically active substances derived from the precursor substance are bound to mucopolysaccharide macromolecules (M), and it is emphasized that one macromolecule M can bind a number of different receptors.

Receptors A, B, H, and Le^b, and the Magard receptor can also be bound to an already formed Le^x – M (Le^a substance). When Le^b, or the Magard receptor, is bound to Le^a substance, the Le^a receptor is lost, being converted into Le^{b_2} receptor, and the Magard receptor is completely lost.

ACKNOWLEDGEMENT

My thanks are due to R. Grubb, R. R. Race, Ruth Sanger, and W. T. J. Morgan for discussions during the preparation of the manuscript.

REFERENCES

1. Andersen, A. 1952. Investigations in the inheritance of the characters secretor and non-secretor. *Acta Path. Microbiol. Scand.* 31: 448–461.
2. Andersen, Jørgen 1958. Modifying influence of the secretor gene on the development of the ABH substance. *Vox Sang.* 3: 251–261.
3. Andersen, Jørgen 1959. On the genetics of the Lewis system. *Acta Path. Microbiol. Scand.* 47: 445–448.
4. Andresen, P. H. 1948. Blood group with characteristic phenotypical aspects. *Acta Path.* 24: 616–618.
5. Andresen, P. H. 1948. The blood group system L.—a new blood group L_2.—A case of epistasy within the blood groups. *Acta Path.* 25: 728–731.
6. Andresen, P. H. 1948. The blood group system L (Lewis), Estatto dalla "*Rivista Dell' instituto Sieroterapico Italiano*". 23: 362–367.
7. Andresen, P. H., and Jordal, K. 1949. An incomplete agglutinin related to the L. (Lewis) system. *Acta Path.* 26: 636–638.

8. ANDRESEN, P. H., ANDERSEN, A., JORDAL, K., AND HENNINGSEN, K. 1950. Corrélation entre le système Lewis et le système sécreteur-non-sécréteur. *Rev. Hémat.* 5: 305–314.
9. ANDRESEN, P. H. 1956. The human blood groups as a biological phenomenon. *Zentr. Bakteriol.* 165: 486–494.
10. ARMSTRONG, C. N., GRAY, J. N., RACE, R. R., SANGER, RUTH, AND THOMPSON, R. B. 1957. A case of true hermaphroditism. *Brit. Med. J.* ii: 605–606.
11. BHENDE, Y. M., DESHPANDE, C. K., BHATIA, H. M., SANGER, RUTH, RACE, R. R., MORGAN, W. T. J., AND WATKINS, WINIFRED 1952. A "new" blood-group character related to the ABO system. *Lancet* i: 903.
12. BIANCO, J., SILVESTRONI, E., LAWLER, S., MARSHALL, R., AND SINISCALCO, M. 1960. Further contributions to the study of Lewis and secretor characters. *Vox Sang.* 5: 337–346.
13. BRENDEMOEN, O. J. 1950. Further studies of agglutination and inhibition in the $Le^a - Le^b$ system. *J. Lab. & Clin. Med.* 36: 335–341.
14. CEPPELLINI, R., NASSO, S., AND TECILAZICH, F. 1952. *La Malattia Emolitica del Neonato.* Instituto Sieroterapico Milanese Serafino Belfanti Milano, p. 204.
15. CEPPELLINI, R. 1955. On the genetics of secretor and Lewis characters: A family study. *Proc. 5th Intern. Congr. Blood Transf. Paris.* 207–211.
16. CEPPELLINI, R. 1959. Physiological genetics of human blood factors. Ciba Foundation Symp. on *Biochemistry of Human Genetics*, 242–260.
17. GAMMELGAARD, A. 1942. Om sjaeldne svage A-receptorer (A_3, A_4, A_5 og A_x), Copenhagen: *Arnold Busck* pp. 137.
18. GLYNN, L. E., AND HOLBORROW, E. J. 1959. Distribution of blood-group substances in human tissues. *Brit. Med. Bull.* 15: 150–153.
19. GRUBB, R. 1948. Correlation between Lewis blood group and secretor character in man. *Nature* 162: 933.
20. GRUBB, R. 1950. Observations on the human group system Lewis. *Acta Path.* 28: 61–81.
21. GRUBB, R. 1953. Zur Genetik des Lewis-Systems. *Naturwissenschaften* 21: 560–561.
22. GRUBB, R. 1955. A note on anti-Le^b and reagents predominantly reacting with group O cells. *Am. J. Phys. Anthrop.* 13: 663–666.
23. GRUBB, R. 1949. The Lewis blood group characters of erythrocytes and body-fluids. *Brit. J. Exp. Path.* 30: 198–208.
24. HARTMANN, G. 1941. *Group Antigens in Human Organs.* Copenhagen: Munksgaard, p. 172.
25. JORDAL, KELL 1957. The Lewis blood group Le^a in adults. *Danish Med. Bull.* 4: 210–217.
26. JORDAL, K. 1956. The Lewis blood groups in children. *Acta Path. Microbiol. Scand.* 39: 399–406.
27. JORDAL, K. 1958. The Lewis factors Le^b and Le^x and a family series tested by anti-Le^a, anti-Le^b and anti-Le^x. *Acta Path. Microbiol. Scand.* 42: 269–284.
27a. JORDAL, K. AND LYNDRUP, S. 1952. The distribution of C-D and Le^a in 1000 mother-child combinations. *Acta Path. Microbiol. Scand.* 31: 476–480.
28. LEVINE, P. AND CELANO, M. 1960. The antigenicity of Lewis (Le^a) substance in saliva coated on to tanned red cells. *Vox Sang.* 4: 53–61.
29. LOGHEM, J. J. VAN AND HART, MIA VAN DER 1955. The weak antigen A_4 occurring in the offspring of group O parents. *Vth Int. Congr. of Blood Trans.* 166–172.
30. MILLER, E., ROSENFIELD, R. E., VOGEL, P., HABER, GLADYS, AND GIBBEL, NATHALIE 1954. The Lewis factors in American Negroes. *Am. J. Phys. Anthrop.* 12: 427–443.
31. MORGAN, W. T. J. 1959. Some immunological aspects of the products of the human blood group genes. Ciba Found. Symp. on *Biochemistry of Human Genetics.* 194–216.
32. MORGAN, W. T. J. 1960. The Croonian Lecture. A contribution to human biochemical genetics; the chemical basis of blood group specificity. *Proc. Royal Soc.* 151: 308–347.
33. MORGAN, W. T. J. AND WATKINS, WINIFRED M. 1959. Some aspects of the biochemistry of the human blood group substances. *Brit. Med. Bull.* 15: 109–112.

34. MOURANT, A. E. 1946. A "new" human blood group antigen of frequent occurrence. *Nature* 158: 237.
35. MÄKELÄ, O. AND MÄKELÄ, PIRJO 1956. Le^b antigen. Studies on its occurrence in red cells, plasma and saliva. *Ann. Med. Exp. Fenn.* 34: 157–162.
36. RACE, R. R. AND SANGER, RUTH 1958. Some modifications of the ABO groups. *Am. J. Clin. Path.* 29: 515–524.
37. SANGER, RUTH 1952. A relationship between the secretion of the blood group antigens and the presence of anti-O or anti-*H* in human serum. *Nature* 170: 78.
38. SCHIFF, F. AND SASAKI, H. 1932. Der Ausscheidungstypus ein auf serologischem Wege nachweisbares mendelndes Merkmal. *Klin. Wchschr.* 34: 1426–1429.
39. SIMMONS, R. T. AND D'SENA, G. W. L. 1955. Anti-H in group O blood. *J. Indian Med. Ass.* 24: 325–327.
40. SNEATH, JOAN S. AND SNEATH, P. H. A. 1955. Transformation of the *Lewis* groups of human red cells. *Nature* 176: 172.
41. SNEATH, JOAN S. AND SNEATH, P. H. A. 1959. Adsorption of blood-group substances from serum on to red cells. *Brit. Med. Bull.* 15: 154–157.
42. WATKINS, WINIFRED M. 1959. Some genetical aspects of the biosynthesis of human blood group substances. Ciba Found. Symp. on *Biochemistry of Human Genetics*. 217–238.
43. WATKINS, WINIFRED M. AND MORGAN, W. T. J. 1957. Specific inhibition studies relating to the Lewis blood-group system. *Nature* 180: 1038–1040.
44. WATKINS, WINIFRED M. AND MORGAN, W. T. J. 1959. Possible genetical pathways for the biosynthesis of blood group mucopolysaccharides. *Vox Sang.* 4: 97–119.
45. WEINER, W., LEWIS, H. B. M., MOORES, PHYLLIS, SANGER, RUTH, AND RACE, R. R. 1957. A gene *y* modifying the blood group antigen A. *Vox Sang.* 2: 25.
46. WIENER, A. S., WEXLER, I. B. 1958. *Heredity of the Blood Groups*. New York: Grune & Stratton, p. 35.

Cancer Rates in Aging Twins*

LISSY F. JARVIK AND ARTHUR FALEK

Department of Medical Genetics, New York State Psychiatric Institute, Columbia University, New York 32, New York

THE PROGRESSIVE EXTENSION OF THE HUMAN LIFE SPAN has been accompanied by an increase in the incidence of malignant neoplastic disease, so that cancer in the broadest sense is now the second leading cause of death after age 40. Despite a variety of theories, there is still a lack of knowledge concerning carcinogenesis in general and the role of genetic factors in particular.

One hypothesis has it that the disorganized and unregulated growth represents a general tissue reaction to noxious agents. Others stress the importance of specific interactions between certain cellular variants and extrinsic factors. In the latter category, it is the role of viruses, or specific DNA particles, which is receiving more and more attention in the etiology of malignant neoplasia (Bendich, in press).

A related issue confronting investigators of this problem concerns the influence exerted by hereditary factors in protection from or susceptibility to the development of malignant growths. Among recent reviews of genetic studies of human cancer have been the following: Jacobsen, 1946; Clemmesen, 1949; Murphy, 1952, 1959; Woolf, 1955; Anderson, Goodman, and Reed, 1958; Graham and Lilienfeld, 1958; and Oliver, 1960. Essentially, three methods of investigation have been employed in this area: (1) pedigree studies, (2) statistical family studies and (3) twin studies.

Pedigree studies served to establish the hereditary basis of retinoblastoma and multiple intestinal polyposis, as well as the precancerous nature of the latter (Franceschetti and Bischler, 1946; Falls and Neel, 1951; Dukes, 1952; Macklin, 1960). By and large, however, the value of numerous published pedigrees showing a high familial incidence of various types of cancer is limited by the inherent weaknesses of the method. These shortcomings include biased reporting, difficulties in obtaining accurate information on members of previous generations, and the possibility "that such families represent chance accumulations or the effect of biological or environmental factors not present in the families of most cancer patients. . . ." (Anderson, Goodman, and Reed, 1958, p. 5).

Family control studies overcome the methodological deficiencies implicit in pedigree investigations. Studies of this kind compare the frequencies of neoplastic disease in relatives of affected index cases with those of non-affected controls. With this family-control technique, most of the recently published reports have shown a greater incidence of neoplastic disease in the relatives of

Received June 12, 1961.

* Presented at the annual meeting of the American Society of Human Genetics in Atlantic City, May 5, 1961.

index cases than in the relatives of controls. This finding applies particularly to cancers of the same type (i.e. at the same site) as that of the index case and to a lesser extent to cancer at all sites.

Statistical studies of families differ in the techniques employed for the ascertainment of index cases, in the definition of matching controls, and in the verification of clinical data on the relatives. The penetrating critique by Graham and Lilienfeld (1958) of the methodology used in such family studies helps to explain that the role of genetic factors in the etiology of malignant neoplasia is still controversial.

The *twin study* approach avoids the difficulty encountered in matching control with patient population. The cotwins of index cases are matched automatically for age, cultural background and socioeconomic factors, and otherwise tend to have fairly similar general environments. In addition, one-egg twins carry the identical complement of genes, while two-egg pairs are genetically no more alike than ordinary brothers and sisters.

The few twin studies that are on record (Macklin, 1940; v. Verschuer and Kober, 1940, 1956; Busk et al., 1948; Nielsen and Clemmesen, 1957; Harvald and Hauge, 1956, 1958) have shown *low* concordance rates ranging from 6.5 to 13.5 percent for one-egg pairs and from 2.8 to 5.3 percent for two-egg twins (except for the 18.8 percent reported by v. Verschuer and Kober for two-egg pairs). Yet, concordance rates for monozygotic twins tend to be twice as high as those for dizygotic pairs. This trend (again with the exception of v. Verschuer and Kober) has been so consistent as to suggest the operation of some genetic influence in determining a certain degree of resistance to malignant neoplasia. In view of the unanimity of investigators concerning the greater similarity in type and localization of lesion betwen monozygotic than between dizygotic members of concordant pairs, the hereditary influence may well consist of several specific factors. To date then, twin studies have done little more than support the findings of family studies.

High among the complicated procedural requirements to be met in twin studies of cancer rank the ascertainment of a representative series of index pairs and the avoidance of biases of selection. Also, final evaluation of twin data is hampered by the lack of readily available vital statistics. It has been suggested by Graham and Lilienfeld that "increased concordance among monozygotics might merely reflect a greater frequency of the disease among monozygotics than dizygotics, in general" (1958, p. 946). There is definite hope that with the completion of the Danish twin investigations by Harvald and Hauge, much of this uncertainty will be erased.

In the meantime, the present report has been prepared to utilize our longitudinal data collected on senescent twins in New York State since 1946 for the purpose of long-range geriatric investigation. A detailed description of these 1603 twin index cases over age 60 (850 alive, 753 deceased) with respect to zygosity, sex, and survival trends was given elsewhere (Jarvik et al., 1960). Briefly stated, an index case was defined as a person over age 60, of multiple birth, residing in or near New York State.

The cancer probands were obtained by a systematic search of the medical histories of all 1603 index cases for any mention of neoplastic disease. Initially, it was planned to survey benign as well as malignant lesions. However, it soon became clear that complete reporting and histopathological verification of lesions were beyond reach for benign tumors. Therefore, the study was restricted to malignant growths. Information provided by twins and relatives was compared with hospital records, reports from private physicians, and official death certificates. The same procedure was employed for data which accrued during the 12-year follow-up of these twins. Additional twin cases came from other departmental research projects dealing with psychotic, tuberculous, or mentally defective twins as well as from the cotwins of senescent index cases, who did not themselves qualify as index cases (failure to survive until age 60 or to fulfill residency requirements).

Inasmuch as only the first group, i.e. senescent index twins, is derived from a well defined population, rigid segregation of the two groups has been maintained throughout this report. While data for the second group are presented for whatever general interest they may have, all essential statistical procedures have been applied only to senescent twin index cases. In other words, the terms *index case* or *cancer proband* refer to those cancer cases that have been ascertained in senescent index twins. The term cancer is used in the broadest sense, that is, for any kind of malignant neoplastic disease.

The statistical information is presented in two sections under the following headings:

1. *Death certificate group*, where the diagnosis of cancer is based exclusively on death certificates, regardless of confirmation.

2. *Verified group*, where the cancer diagnosis has been confirmed by clinical or pathological means, regardless of the information provided by death certificates. This group includes surviving as well as deceased index twins.

1. DEATH CERTIFICATE GROUP

From our data on senescent twin index cases it is possible to compute cancer rates in twins over age 60 and compare them with rates for the general population. Since general population statistics are based on unverified death certificate information, the same procedure had to be followed in analyzing our twin data.

Cancer was listed among the causes of death for 68 index twins or 9.0 percent of the 753 deceased twin index cases. This frequency was significantly lower than the 16.5 percent reported for the New York State white population over age 60. Plausible explanations for this discrepancy include changes in cancer death rates during the past twenty years and dissimilarities in the age and sex distribution of twins and the general population. For this reason cancer death rates were computed separately for males and females in three age groups (60–69, 70–79, and 80 and over) as shown in table 1. Corresponding rates for the New York State white population were derived from vital statistics for 1939 and 1958.

Evidently, cancer rates have increased continuously since 1939 for both

TABLE 1. CANCER DEATHS AS PERCENT OF TOTAL DEATHS AFTER AGE 60

New York State White Population

	1939	1958	Average 1939 & 1958	Senescent Index Twins
Males				
60–69	17.2	21.2	19.8	8.7 (10)*
70–79	14.9	17.9	16.5	12.6 (19)
80+	8.5	10.4	9.7	3.6 (3)
Females				
60–69	19.6	25.2	22.5	8.2 (8)
70–79	13.7	15.5	14.7	12.2 (18)
80+	7.9	8.1	8.0	6.2 (10)

* Number of twins given in parentheses

males and females in all three age groups. Of the 68 index twins whose cancer diagnosis was based solely on death certificates, 28 died between 1949 and 1958, 42 between 1939 and 1948 and only eight prior to 1939. According to these figures (table 1), the frequency of cancer as a cause of death (given as percentage of all deaths) is lower for twins than for the corresponding general population in all age groups and in both sexes, especially for the age group 60–69. The consistency of this trend would seem to indicate that cancer, as a cause of death after age 60, is less frequent in twins than in the general population. The validity of this conclusion is supported by the results of a previous analysis (Falek et al., 1960), based on the life spans of the 753 deceased senescent twin index cases, which demonstrated that the total death rate of the twins was no greater than that of the general population over age 60. Indeed, there was a tendency for twins to show a slightly longer span of life, which if true, would strengthen the inference that cancer deaths occur less often in twins over age 60 than in the general population.

The relatively low cancer death rate observed in aging twins is not in line with the belief that twinning itself represents a tendency toward tumor formation (Macklin, 1940). Since this statement applies only to monozygotic twins, a breakdown by zygosity is given for one-egg and same sex two-egg pairs in table 2.

Comparison of the two groups reveals no difference between them and justifies the assumption that, above the age of 60, cancer is as frequent a cause of death in dizygotic as in monozygotic twins. Only 12 of the 68 twins (18 percent) in the death certificate group were members of opposite sex pairs. This figure is considerably lower than that observed in the parent sample where opposite sex

TABLE 2. CANCER DEATH RATES IN SAME SEX SENESCENT INDEX TWINS

(Death Certificates Regardless of Verification)

	Number of Cancer Twins			Percent of Total Deaths		
	Male	Female	Total	Male	Female	Total
One-Egg	12	13	25	9.0	9.5	9.2
Two-Egg	12	13	25	10.5	8.2	9.2*

* Opposite sex twins 8.8 percent; unclassified twins 9.1 percent

TABLE 3. TUMOR SITES OBSERVED AND EXPECTED IN 68 DECEASED SENESCENT TWIN INDEX CASES ACCORDING TO CANCER DIAGNOSIS ON DEATH CERTIFICATE (REGARDLESS OF CONFIRMATION)

	Male		Female	
	Observed	Expected	Observed	Expected
Breast	0	0.1	7	6.0
Digestive System	13	14.5	21	16.1
Respiratory	6	7.3	0	1.8
Genital	5	3.3	5	5.1
Urinary	1	2.3	0	1.4
Leukemia-Lymphoma	4	2.1	1	2.3
Others	3	2.4	2	3.2
Total	32	32.0	36	35.9

pairs formed 27 percent of the 1603 senescent twin index cases. Since twins were considered alive as of the date of last information, the less complete follow-up of opposite sex than of same sex pairs, described elsewhere (Jarvik et al., 1960), may account for the given discrepancy. This interpretation is borne out by the fact that of the 753 deceased index twins only 18 percent belonged to opposite sex pairs.

The similarity in the cancer rates for one-egg and two-egg twins is particularly striking when it is recalled that these are unselected pairs, representing all those among the 753 deceased index twins whose official death certificates contained a cancer entry (any malignant neoplastic disease). For data obtained in this fashion the cancer frequencies appear to be independent of zygosity (ranging from 8.8 and 9.1 percent for opposite sex and unclassified cases respectively to 9.2 percent for one-egg and same-sex two-egg twins). No comparable twin data are available to confirm these findings.

A similar situation exists in relation to tumor localization, and for that reason vital statistics have again been used. The tumor sites observed in the "death certificate group" of 68 index twins are listed in table 3 and show fairly good agreement with the expected values calculated from New York State vital statistics for 1958. It may be inferred, therefore, that site-specificity in twins approximates that in the general population, at least for the most prevalent forms of cancer.

One possible explanation for the observed deficiency of cancer deaths in twins over age 60 may be seen in the greater antenatal and neonatal mortality of twins compared with singletons. It has been postulated that, as a result of this process of selection, surviving twins have greater constitutional resistance than single-born populations. This hypothesis may be extended to include selection for some general protective factors against neoplastic disease, although it has not been substantiated by factual evidence. It would also be difficult to assume that influences operating at the time of birth tend to confer a specific immunity to the subsequent development of cancer.

Equally weak is the theory that neoplastic disease may affect twins at an earlier age than singletons. In this case, death from cancer should be correspond-

ingly higher in twins than in the general population for ages below 60 years. Corroborative data are entirely lacking.

More plausible would be the interpretation attributing to artefacts the apparent discrepancy in cancer mortality rates between twins and the general population. Death certificates are known to lack reliability and it is possible that, in the absence of adequate documentation, physicians are reluctant to make the diagnosis of cancer in cases with a known surviving cotwin. Such special considerations would operate less frequently in single-born patients.

As mentioned before, the decision to use death certificates was prompted by the need for comparing data on twins and the general population, despite the well-known shortcomings of such information. Another imperfection in this method is the automatic exclusion of any cancer twins who are still alive. These pitfalls have ben avoided in the approach described below.

2. VERIFIED GROUP

At the time of analysis, our cancer roster consisted of 161 twins with alleged neoplastic disease. Senescent twin index cases provided 117 of them and the remaining 44 accrued from other departmental twin projects. Every effort was made to secure medical documentation of the cancer history in each case, especially pathological reports on biopsy and autopsy material. While the review by our pathologist of all available evidence is still in progress, it has been completed for questionable diagnoses and for all concordant pairs. Cases without pathological confirmation (either for want of surgical exploration, failure to obtain biopsy material, or lack of autopsy) were accepted only on the basis of classical X-ray, laboratory, or clinical findings. Rigid adherence to these criteria is reflected by a large number of rejected cases. As shown in table 4, 49 of 117 index cases and 18 of 44 other twins were classified as "not acceptable", constituting 42 and 41 percent of the respective groups. Benign tumors (5 cases) or tuberculous lesions (2 cases) were diagnosed in seven of the 49 index twins, and the remainder were rejected because of uncertainties as to the nature of their lesion. Only 17 index twins (14 percent) without pathological confirmation were included as "clinically acceptable", while 51 others (44 percent) fell into the category of "pathologically confirmed" twin index cases.

The rejection rate for two-egg twins was about twice that for one-egg twins, both in the male (48 vs. 24 percent) and female groups (58 vs. 30 percent). Apparently, intra-pair differences in survival and inter-twin relationships were among the factors favoring one-egg twins in the collection of confirmatory evidence and the discrepancy in the rejection rate may be in part a function of strict requirements for the diagnosis of cancer.

Conservatism is also responsible for the designation of "probable" cases (table 4), while all twins with serious doubt concerning zygosity were placed in the "unclassified" category (7.7 percent of index twins). The term "probable" refers to zygosity diagnoses which were considered well-founded but lacked corroborative documentation, usually because of the death of one twin and the consequent necessity to rely upon interviews. In the various calculations, "proba-

TABLE 4. SURVEY OF 161 TWINS WITH REPORTED NEOPLASTIC DISEASE

Zygosity	Senescent Twin Index Cases			Other Twins with Tumor History			
	Pathologically Confirmed	Clinically Acceptable	Not Acceptable	Pathologically Confirmed	Clinically Acceptable	Not Acceptable	Total
Male							
One-egg	10	3	4[1]	1	1	—	19
Two-egg	6[1*]	3	6	1	—	3	19
Opposite sex	1	1	4	1	1	—	8
Unclassified	5	—	1	2	—	3	11
Female							
One-egg	15[1]	4	8[2]	8[3]	1	1	37
Two-egg	9[1]	2[1]	18[4]	4[2]	1	4	38
Opposite sex	3	4	7	2	2	3	21
Unclassified	2	—	1	1	—	4	8
Total	51	17	49	20	6	18	161

* The superscript corresponds to the number of twins in each group with "probable" rather than positive zygosity diagnosis

ble" cases have been combined with definite ones but they have been identified in such a manner that they can be omitted if so desired. The issue concerns only four of the 68 index cases with verified cancer.

These 68 cases consist of 51 pathologically and 17 clinically confirmed cases. It may be stressed, however, that although the total numbers of the two groups ("death certificate" and "verified") happen to be identical, the cases themselves are *not* the same. There is, of course, considerable overlap between the groups in that 48 twins from the "death certificate group" also fall into the "verified group". The following reasons led to elimination of the other 20: Pathologically no cancer, two cases (death certificate filed prior to autopsy report); no information from hospitals or family physicians to confirm death certificate, ten cases; hospital records unclear, at variance with those of private physician, or diagnosis not definitive, eight cases. The 20 index twins whose cancer diagnosis did not appear on death certificates were distributed as follows: Still alive, nine cases; cancer apparently cured and not a cause of death, eight cases; cancer diagnosed post mortem, one case; reason for omission from death certificate unknown, two cases. Pathological confirmation has been obtained for 19 of these 20 additional cases.

As an approximate measure of the accuracy of death certificate information, we may consider errors of commission (two cases where autopsies refuted cancer diagnosis) and omission (three cases with pathologically established cancer diagnosis) as a fraction of the 53 cases for which the diagnosis could either be proven or disproven. The given 9.4 percent provide a lower limit for the estimate of error, because the likelihood of error is considerably higher for the 18 cases which had to be omitted for lack of definitive information. Nevertheless, death certificates may be more useful than other sources for some special analyses by

TABLE 5. CANCER DEATH RATES IN SAME SEX SENESCENT INDEX TWINS
(Cancer Verified Cause of Death)

	Number of Cancer Twins			Percent of Total Deaths		
	Male	Female	Total	Male	Female	Total
One-Egg	12	11	23	9.0	8.0	8.5
Two-Egg	9	6	15	7.9	3.8	5.5*

* Opposite sex twins 5.2 percent; unclassified twins 9.0 percent

virtue of the fact that random distribution of errors may be assumed for such variables as zygosity. By contrast, imposition of rigorous diagnostic standards may produce differential effects upon data for one-egg and two-egg pairs as shown by variations in the rejection rate.

This reservation applies particularly to countries like the United States where general population registers are lacking. Conceivably, the figures in table 5 may exemplify a spurious difference introduced in this manner. Contrary to the data which were based on unconfirmed death certificate diagnoses and yielded equal cancer death rates for the two zygosity groups (table 2), the findings derived from 59 deceased index twins with verified cancer show far lower frequencies for two-egg than one-egg pairs. Removal of the nine cases whose deaths were apparently unrelated to cancer increases this difference in the female group. Intriguing as these contradictions may be, they lack statistical significance, and their resolution may have to wait for the investigation of Danish twins now in progress. With complete population registers at their disposal in a country where post-mortem examinations are the rule and not the exception, Harvald and Hauge may be able to collect data without such intervening variables as have an effect on American twin studies.

SUMMARY AND CONCLUSIONS

As part of a longitudinal study of aging, cancer data accrued on 1603 twin index cases over age 60 in New York State. At the time of the last analysis, 850 twins were recorded as living and 753 as deceased.

Death certificates for the 753 deceased index twins yielded 68 cases in which cancer (broadly defined as malignant neoplastic disease) was listed among the causes of death. Cancer death rates, based on these 68 cases in the "death certificate group", were consistently and significantly lower than those for the white male and female New York State population of comparable age. Several possibilities have been considered to explain this unexpected finding.

Cancer frequencies were found to be independent of zygosity. It has been pointed out that artefacts of selection may result in spurious differences between the zygosity groups.

It may be stated on the basis of the present study that cancer as a cause of death during senescence appears to be as frequent in monozygotic as in dizygotic twins. Cancer may be less common as a cause of death for twins in this age group than would be expected from general population statistics (9.0 vs. 16.5 percent). A total of 1603 twin index cases in New York State provided

evidence of only 68 verified cases of cancer (59 deceased and 9 surviving) within a 12-year follow-up period. Evidently, studies on an even larger scale are needed for a final evaluation of this finding.

ACKNOWLEDGMENTS

We wish to express our gratitude to Dr. Franz J. Kallmann, Chief of our Department of Medical Genetics and Professor of Psychiatry at Columbia University, for his guidance and inspiration throughout the course of this study; to Dr. W. Edwards Deming, Professor of Statistics at New York University, for assistance in evaluating the statistical findings and for his critical review of the manuscript; to Dr. Leon Roizin, Chief of the Department of Neuropathology, for examination of the histopathological material; and to Mrs. Laine Ruut for her assistance in collecting the data and preparing the final manuscript.

During the earlier phases of the project, financial support was provided by the Rockefeller Foundation (1945–1951) and the National Institutes of Health (1952–1959).

REFERENCES

Anderson, V. E., Goodman, H. O., and Reed, S. C. 1958. *Variables Related to Human Breast Cancer.* Minneapolis: University of Minnesota Press.

Bendich, A. The biological properties of nucleic acid. *Dis. Nerv. Syst.* (in press).

Busk, T., Clemmesen, J., and Nielsen, A. 1948. Twin studies and other genetical investigations in the Danish Cancer Registry. *Brit. J. Cancer* 2: 156–163.

Clemmesen, J. 1949. Status of genetical studies in human cancer. *Brit. J. Cancer* 3: 474–484.

Dukes, C. E. 1952. Familial intestinal polyposis. *Ann. Eugen.* 17: 1–29.

Falek, A., Kallmann, F. J., Lorge, I., and Jarvik, L. F. 1960. Longevity and intellectual variation in a senescent twin population. *J. Gerontol.* 15: 305–309.

Falls, H. F., and Neel, J. 1951. Genetics of retinoblastoma. *Arch. Ophthalmol.* 46: 367–389.

Franceschetti, A., and Bischler, V. 1946. Rétinoblastome et héredité. *Arch. Julius Klaus Stift.* 21: 322–328.

Graham, S., and Lilienfeld, A. M. 1958. Genetic studies of gastric cancer in humans: An appraisal. *Cancer* 11: 945–958.

Harvald, B., and Hauge, M. 1956. A catamnestic investigation of Danish twins. *Danish Med. Bull.* 3: 150–158.

Harvald, B., and Hauge, M. 1958. A catamnestic investigation of Danish twins. *Acta Genet. et Stat. Med.* 8: 287–294.

Jacobsen, O. 1946. Heredity in breast cancer. *Op. dom. Biol. hered. hum., Kbh.* 11: 1–306.

Jarvik, L. F., Falek, A., Kallmann, F. J., and Lorge, I. 1960. Survival trends in a senescent twin population. *Am. J. Human Genet.* 12: 170–179.

Kallmann, F. J. 1961. Genetic factors in aging: Comparative and longitudinal observations on a senescent twin population. In: Hoch and Zubin [eds.] *Comparative Epidemiology of Mental Disorders.* New York: Grune & Stratton.

Macklin, Madge T. 1940. An analysis of tumors in monozygous and dizygous twins. *J. Hered.* 31: 277–290.

Macklin, Madge T. 1960. Genetic considerations in human breast and gastric cancer. In: *Genetics and Cancer.* Austin: University of Texas, pp. 409–425.

Murphy, D. P. 1952. *Heredity in Uterine Cancer.* Cambridge: Harvard University Press.

Murphy, D. P., and Abbey, H. 1959. *Cancer in Families. A study of the Relatives of 200 Breast Cancer Probands.* Cambridge: Harvard University Press.

Nielsen, A., and Clemmesen, J. 1957. Twin studies in the Danish Cancer Registry, 1942–55. *Brit. J. Cancer* 11: 327–336.

OLIVER, C. P. 1960. Genetic studies of families with high cancer incidence. In: *Genetics and Cancer*. Austin: University of Texas, pp. 427–438.

VERSCHUER, O. v., AND KOBER, E. 1940. Die Frage der erblichen Disposition zum Krebs. *Z. Krebsforsch.* 50: 5–14.

VERSCHUER, O. v., AND KOBEP, E. 1956. Tuberkulose und Krebs bei Zwillingen. *Acta Genet. et Stat. Med.* 6: 103–113.

VITAL STATISTICS OF THE UNITED STATES 1941. (Part 1 – 1939). Washington, D. C., U. S. Government Printing Office, pp. 292–293.

VITAL STATISTICS OF THE UNITED STATES 1960. (Vol. 2 – 1958). Washington, D. C., U. S. Government Printing Office, pp. 442–443.

WOOLF, C. M. 1955. Investigations on genetic aspects of carcinoma of stomach and breast. *Univ. California Publ. in Public Health* 2: 265–349.

BOOK REVIEWS

Cell Physiology of Neoplasia

Fourteenth Annual Symposium on Fundamental Cancer Research, 1960, The University of Texas M. D. Anderson Hospital and Tumor Institute, University of Texas Press, Austin, 1960, 653 pp., $10.50.

THIS ANNUAL SYMPOSIUM at the M. D. Anderson Hospital and Tumor Institute is generally considered by cancer investigators as one of the most outstanding meetings on cancer research each year. The 1960 meeting reported in this book upheld this reputation.

There were 19 papers in four areas of cellular physiology that are published with excellent illustrations and with the discussion that followed the presentation of each. The first four papers are devoted to the nucleus. Koller discusses chromosome behavior in tumors in relation to the original question posed by Boveri: Is a grossly visible change in chromosome constitution the forerunner of malignancy? He brings out clearly that it is not possible to say whether abnormalities observed in ideograms of transplanted or cultured tumors are related to the neoplastic change, but the chromosome variability in primary neoplasms may have a more direct bearing on the question. Yerganian introduces the triheterosomic scheme for sex determination that he and his coworkers have put forth from their studies on the Chinese hamster and points out how the scheme may be applied to explain the origins of human intersexes and sex reversals. The scheme employs an X_1X_2/X_1Y combination, the X_1 being common to both sexes, the X_2 present in the normal female, and the Y in the normal male. A chapter by Kopac on experimental studies on malignant nucleoli will be of particular value for those carrying genetics into tissue culture for it is strong on micrurgical instrumentation and procedures. In the final chapter of the group, Watson describes his electronmicroscope studies of the nuclear membrane emphasizing the pore complex that is postulated as bringing the elaborate cytoplasm into closer contact with the nucleus enabling the more complex nuclear function to regulate the cytoplasm.

The next section is on the cytoplasm. Dalton discusses his favorite subject, the Golgi complex, and presents his reasons for considering it in the category of omnipresent cell components such as the nucleus, mitochondria, and endoplasmic reticulum. Dmochowski *et al.* describe electron microscope studies of the morphology of cells infected with tumor viruses, particularly the polyoma virus and the myeloblastosis virus of chickens. Novikoff's chapter on enzyme localization in tumor cells will be of special value in its emphasis of methodology—isolation of subcellular particles and staining methods for enzyme activities. The section is concluded with a chapter by Caspersson *et al.* on techniques of quantitative cytochemical studies of the cell with the use of microspectrography, microradiography, and microinterferometry.

The nucleic acids command the third section. The Leuchtenbergers discuss DNA in relation to tumor cells and virus infections. Kit discusses RNA presenting the evidence that it is a single stranded coil and discusses evidence for the role of nuclear RNA as a precursor of cytoplasmic RNA. Beck has a chapter concerned with the possible relationship between cancer and the biochemistry of RNA and its precursors. A discussion by Colter and Ellem on ribonucleic acids of viral origin concludes the papers on nucleic acids.

The final section on cell growth and cell development includes a chapter by Chang on

his enzymatic studies of hepatic cells in the early stages of carcinogenesis, one by Duryee on the physiology of the nucleus as basic in our understanding of cancer, one by Harris and Ruddle on the individuation of somatic cell types in vivo with development of drug resistance as a model for study, a chapter very basic to genetics by Taylor on duplication of chromosomes, one by Kinosita and Ohno on mitosis and cyto-differentiation, and one by King and McKinnell on the developmental potentialities of the cancer cell nucleus as revealed through transplantation studies. Dr. E. V Cowdry, a long established cancer investigator, received the Bertner Award. His lecture on The Lives of Cancer Cells is included.

With his broad knowledge of the general topic, Kopac concludes the symposium with a thoughtful summarizing chapter on problems and perspectives. It is hoped that in this chapter he will have succeeded in driving home the reality presently often overlooked that the road to solutions of the cancer problem is through fundamental knowledge of cellular biology.

W. E. Heston
National Cancer Institute
National Institutes of Health
Bethesda 14, Maryland

The Processes of Ongoing Human Evolution.

Edited by Gabriel W. Lasker. Detroit: Wayne University Press, 1960, 108 pp., $3.75.

In the days when races were regarded as rather fixed "types," of long standing and slow change, the possible processes of change (mixture excepted) were almost ignored. They were known only from some classical studies of race mixture, which showed that brachycephaly might or might not be dominant over dolichocephaly, and so forth; and from work like that of Boas indicating that racial "types" were not quite as immutable as statuary, but could in fact change slightly in size or form as they changed their skies or their centuries. Then about 1940 genetics hit the anthropologists almost like a new religion. In fact, conversion seemed easy to many, consisting of a recital of Mendel's laws and an avowal that blood groups are better than head measurements. Joking aside, however, the exceedingly productive exploitation of serology did not reveal very clearly why genetics was important to anthropology; the blood groups merely appeared to show that Mendel's laws did indeed apply to man, and in traits which were non-pathological and non-adaptative, i.e., as simple polymorphisms in the population.

It is really very recently that the significance of the last aspect has sunk in, and the notion of non-adaptiveness seen to be both a mistake and a hindrance. The symposium represented by the book under review recognizes this completely, and clearly if not explicitly takes "polymorphism", with all the term implies, as the keynote. The papers are by young and not-so-old anthropologists, and in several ways they show how we now may study the bases of adaptation and micro-evolution using actual human (i.e. anthropological) materials instead of having to borrow from other biologists. They are well organized and each limits its scope to a useful point. Baker deals with the surface-area weight ratio as an adjustment to climate and temperature, more critically than previous writers, and with consideration of the role of culture in such human adjustments. Livingstone treats ABO antigens and disease resistance, again not as previous writers have but from the

viewpoint of a mathematical model of balanced fitnesses in a whole disease picture, i.e. hypothetically. Motulsky's paper is a different view of similar ideas, suggesting that past histories of infectious diseases have been responded to in continental populations by varied chemical and enzymatic polymorphisms (drawing attention to the powerful evidence of these as genetic adaptations which is provided by the lack of genetic immunity of previously isolated populations—e.g. Indians or Polynesians—to new diseases—e.g. measles.) Hulse considers phenotypic plasticity in human size and form, and Lasker some of the social processes of migration and mating patterns.

Finally, Reynolds steps into a more public arena with an attack on complacency over man-made radiation. His paper is strongly phrased and a somewhat personal document, doubtless what more scientists should be indulging in. But it belongs in another symposium. The remaining papers in this one join very successfully in indicating where we stand in the study of actual evolution in human beings. We know almost nothing, it is true, but this well written series shows that we have a posture of approach, and are faced in the right direction for some immediate useful work.

W. W. Howells
Harvard University

LETTER TO THE EDITOR

Reprinted from *Lancet*, Vol. 1: 775 (Apr. 8) 1961.

Mongolism

It has long been recognized that the terms "mongolian idiocy", "mongolism", "mongoloid", etc., as applied to a specific type of mental deficiency have misleading connotations. The occurrence of this anomaly among Europeans and their descendents is not related to the segregation of genes derived from Asians; its appearance among members of Asian populations suggests such ambiguous designations as, "mongol Mongoloid"; and the increasing participation of Chinese and Japanese investigators in the study of the condition imposes on them the use of an embarrassing term. We urge, therefore, that the expressions which imply a racial aspect of the condition be no longer used.

Some of the signers of this letter are inclined to replace the term "mongolism" by such designations as "Langdon-Down anomaly", or "Down's syndrome or anomaly" or "congenital acromicria". Several other signers believe that this is an appropriate time to introduce the term "trisomy 21 anomaly" which would include cases of simple trisomy as well as translocations. It is hoped that agreement on a specific phrase will soon crystalize if once the term "mongolism" has been abandoned.

GORDON ALLEN (*Bethesda, Md., USA*)
C. E. BENDA (*Waverly, Mass., USA*)
J. A. BÖÖK (*Uppsala, Sweden*)
C. O. CARTER (*London, England*)
C. E. FORD (*Harwell, England*)
E. H. Y. CHU (*Oak Ridge, Tenn., USA*)
E. HANHART (*Ancona, Switzerland*)
GEORGE JERVIS (*Letchworth Village, New York, USA*)
W. LANGDON-DOWN (*Normansfield, England*)
J. LEJEUNE (*Paris, France*)
HIDEO NISHIMURA (*Kyoto, Japan*)
J. OSTER (*Randers, Denmark*)
L. S. PENROSE (*London, England*)
P. E. POLANI (*London, England*)
EDITH L. POTTER (*Chicago, Ill., USA*)
CURT STERN (*Berkeley, Calif., USA*)
R. TURPIN (*Paris, France*)
J. WARKANY (*Cincinnati, Ohio, USA*)
HERMAN YANNET (*Southberry, Conn., USA*)